Canadian
Journal of Chemistry

PYROLYSIS OF ETHYL MERCAPTAN[1]

BY JEAN L. BOIVIN AND RODERICK MACDONALD

ABSTRACT

The decomposition of ethyl mercaptan to ethylene and hydrogen sulphide was studied at various temperatures, with and without a catalyst. Metal sulphides (copper, nickel, and cadmium) proved to be the most efficient catalysts for cracking ethyl mercaptan into unsaturated end products, the optimum temperature being 500–600° C. When no catalyst was used a 40–50% yield of ethylene and a nearly quantitative conversion to hydrogen sulphide was observed between 600 and 700° C. Other products identified in the exit gas were carbon disulphide, carbonyl sulphide, methane, hydrogen, ethane, thiophene, diethyl sulphide, and free sulphur. Identification of these products was aided by infrared and mass spectral analysis of the gas. A tentative mechanism for the reaction justifying the presence of the above by-products is outlined.

INTRODUCTION

Sabatier and Mailhe (4) report that diethyl sulphide and hydrogen sulphide are formed when dry ethyl mercaptan is passed over a cadmium sulphide catalyst at 330° C. and that upon heating to 380° C. ethylene and hydrogen sulphide are quantitatively formed:

$$2CH_3CH_2\text{—}SH \xrightarrow[\text{CdS}]{330°\,C.} (CH_3CH_2)_2S + H_2S \qquad [1]$$

$$CH_3CH_2\text{—}SH \xrightarrow[\text{CdS}]{380°\,C.} CH_2\text{==}CH_2 + H_2S. \qquad [2]$$
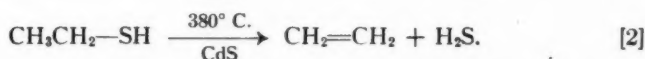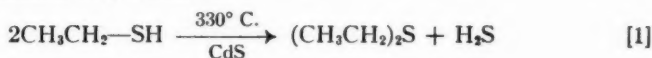
The conditions reported by Sabatier and Mailhe were reproduced but no reaction product was obtained. Hydrogen sulphide was absent, which proved that even diethyl sulphide was not formed (Eq. [1]) at this temperature. A study was then undertaken to determine the optimum conditions for the preparation of ethylene and hydrogen sulphide from ethyl mercaptan.

APPARATUS

The reaction chamber consisted of a Pyrex or silica tube 24 in. in length and 1 in. in diameter mounted vertically in a cylindrical furnace. The bottom of the tube was connected through a ground-glass joint to a two-necked flask which served as the vaporization vessel. To the second neck was attached a measuring dropping funnel for the introduction of ethyl mercaptan. The products of the reaction were allowed to escape through an opening at the

top of the reaction chamber and passed through an upright water-cooled condenser. The gaseous fraction was led from the top of the condenser to a series of scrubbers (for H_2S and unsaturated compounds) while condensable materials were retained in a flask attached to the bottom of the condenser. In operation the reaction tube was packed with alternate layers of glass wool and catalyst, or glass wool only. The inside temperature was recorded by means of a thermocouple. The vaporization flask was heated by a glas-col mantle.

GENERAL METHOD

In operation a weighed quantity of ethyl mercaptan was added from the dropping funnel at the rate of 1 gm. per min. to the vaporization flask which was held at 150°C. The volatilized mercaptan entered the reaction tube (held at a constant temperature) and emerged as a mixture of gases and some condensable products. The gases were bubbled first through water to remove free sulphur, then through 30% sodium hydroxide to remove hydrogen sulphide, and finally through bromine (under water) to remove unsaturated material.

When a total analysis of the reaction products was required a sample was taken directly from the top of the condenser. Using an Orsat apparatus, the sample (100 ml.) was measured at equalized pressure and sent through an absorption pipette containing 30% sodium hydroxide until the volume of remaining gas remained constant. The loss in volume was measured as hydrogen sulphide. Unsaturated materials were removed with fuming sulphuric acid (20%) or bromine. The remaining portion was passed through hot copper oxide several times to determine its hydrogen content, and then the remainder was burned in the presence of oxygen and the volumes of carbon dioxide and water formed were measured. The latter measurements enable one to determine the amount of methane and ethane in a sample of gas when only the two are present.

RESULTS

Since cadmium sulphide was reported as a good catalyst by Sabatier, it was studied over a wide range of temperatures. One mole of ethyl mercaptan was used and samples were taken five to ten minutes after the reaction had begun, to ensure the absence of air.

With this catalyst, formation of ethylene (Table I) started at 400° C. with a yield of only 5.7% of the total gases coming out of the reactor. Also much liquid condensed, which was identified as diethyl sulphide with minor quantities of ethyl mercaptan.

A maximum yield was attained at 600° C. with 24.1% of ethylene in the gas mixture. As can be noted, the ethylene formation passed through a maximum at 600° C. The hydrogen sulphide formation was very high at the start and then decreased steadily with increasing temperatures. Sulphur was also formed in small amounts.

Hydrogen formation increased with the temperature of pyrolysis. This is undoubtedly due to the cracking of hydrocarbon or hydrogen sulphide. Also the mixture of gas B after ethylene, hydrogen sulphide, and hydrogen have

TABLE I

COMPOSITION OF EFFLUENT GASES

Catalyst	Temp., ° C.	C_2H_4, %	H_2S, %	H_2, %	B, %
CdS	300	Nil	Nil	Nil	Nil
	350	Nil	Nil	Nil	Nil
	400	5.7	88.4	Nil	5.9
	450	10.5	75.2	8.5	5.8
	500	16.3	58.7	14.0	11.0
	550	20.3	47.2	17.7	14.8
	600	24.1	44.5	16.9	14.5
	700	17.6	37.4	24.0	21.0
NiS	400	Nil	Nil	Nil	Nil
	450	20.2	64.8	1.0	14.0
	500	28.8	52.1	1.3	17.8
	550	27.0	51.5	1.3	20.2
	600	27.9	48.9	3.2	21.0
	700	26.8	43.0	6.8	23.4
$(Al_2O_3)_x(SiO_2)_y$	400	Nil	Nil	Nil	Nil
	450	29.5	0.5	0.5	2.0
	500	30.3	0.4	0.4	10.0
	550	30.6	0.4	0.4	15.0
	600	25.5	1.3	1.3	21.0
	700	19.6	9.0	9.0	26.0
None	450	Nil	Nil	Nil	Nil
	500	31.9	49.4	0.8	17.9
	550	30.9	49.0	0.9	19.2
	600	29.1	48.5	0.8	21.6
	650	28.1	46.3	2.4	23.2
	700	30.1	45.0	4.1	20.8

B refers to gases that could not be analyzed by the Orsat apparatus.

been removed increased with the temperature. This mixture of gases when burned in the Orsat apparatus could not be calculated as methane and ethane.

Following these results a search for a more efficient catalyst was made. Other sulphides were studied.

With nickel sulphide catalyst (Table I) results were similar to cadmium sulphide except that the formation of ethylene took place at a higher temperature (450° C. instead of 400° C.).

Other sulphides were used, namely those of cobalt, copper, and iron, and gave similar results to nickel sulphide with minor differences.

Aluminosilicate behaved like sulphides, except that less hydrogen was formed. The yield of ethylene was about 30% at 500° C.

Without a catalyst (Table I), ethylene was produced at 500° C. and the composition of the gas remained nearly constant from 500 to 700° C.

IDENTIFICATION OF PRODUCTS

The condensate obtained from the reaction products was quite large at low reaction temperatures. This was shown by a boiling point determination to be diethyl sulphide with a small amount of unreacted ethyl mercaptan.

A white colloidal material was noticed in the water scrubber after the reaction had proceeded for 30 min. Extraction with carbon disulphide removed this material. Upon evaporation sulphur was obtained.

(a) Infrared Spectra

The gas coming out of the reactor was sampled in a gas cell with sodium chloride windows. Another sample was usually taken after hydrogen sulphide and ethylene were removed.

Ethylene was easily identified by its broad and very intense band at 949 cm.$^{-1}$ and a triplet at 1860, 1889, 1910 cm.$^{-1}$ (3). Other bands may be common to other products.

After removal of ethylene and hydrogen sulphide, the spectrum also showed the presence of methane with an absorption band at 1306 cm.$^{-1}$ (3) and of ethane at 820 cm.$^{-1}$ (3).

The mixture also contained carbon disulphide as shown by the band at 2183 cm.$^{-1}$ (3).

Carbonyl sulphide was detected by its very intense absorption at 2050 cm.$^{-1}$, which in many cases has a dual nature under dilution (1). Another band which might be characteristic of thiophene was present at 710 cm.$^{-1}$ (2). The spectrum of the unscrubbed and scrubbed gases is fully explained and all bands were assigned to known gases.

(b) Mass Spectrometry

In order to know the relative amount of product present in one of these mixtures of gases mass spectral analysis was used. Since the Orsat apparatus could give reasonable accuracy for the estimation of ethylene, hydrogen sulphide, and hydrogen, the samples used for mass spectral analysis were free of hydrogen sulphide and ethylene.

Results obtained when using cadmium sulphide catalyst at 500° C. showed an average composition of 41% of ethane, 8.4% of methane, and 47% of hydrogen, and traces of other materials such as carbon disulphide, carbonyl sulphide, and thiophene. Therefore the proportion of ethane in this gas mixture is high and that of methane, low. The composition of this fraction of gas indicates cracking of ethane or ethylene.

DISCUSSION

The pyrolysis of ethyl mercaptan under flow conditions is very slightly catalytic if at all. With the best catalysts used, such as sulphides of copper, nickel, cobalt, and cadmium, the lowest temperature at which ethylene is produced is 450° C. The yield is small, and the best temperature for the optimum formation of ethylene, with catalysts, is 500° C. Moreover, from 500 to 700° C. without a catalyst, the yield of ethylene is practically constant. It is considered that temperatures of 500–600° C. would be efficient using Pyrex or silica tubes packed with glass wool.

MECHANISM

The basic reaction in the pyrolysis of ethyl mercaptan is analogous to the dehydration of ethyl alcohol. This dehydration reaction is reversible under certain conditions. Also the pyrolysis of ethyl mercaptan, which can be termed as a desulphurization process, is a reversible reaction:

$$CH_3CH_2—OH \xrightarrow[H_2SO_4]{Al_2O_3} CH_2{=}CH_2+H_2O \qquad [3]$$

$$2EtOH \longrightarrow Et_2O+H_2O \qquad [4]$$

$$CH_3CH_3SH \rightleftharpoons CH_2{=}CH_2+H_2S . \qquad [5]$$
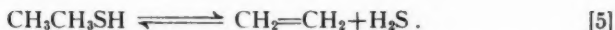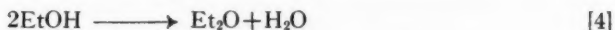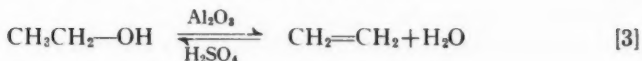
At lower temperatures, when the hydrogen sulphide formation is high, some liquid is formed, which was identified as diethyl sulphide (Eq. [1]).

Since elemental sulphur is formed in the pyrolysis, it should come from the decomposition of hydrogen sulphide which is said to start at 400° C. Even if the dissociation of hydrogen sulphide is small, the equilibrium is shifted to the right by removal of sulphur or hydrogen, which are both present in the reaction products.

Ethane is found at all the temperatures studied as evidenced by the infrared spectra obtained. It is likely to be formed by the reduction of ethylene with the hydrogen generated from hydrogen sulphide. At higher temperatures methane is produced in greater proportion and the hydrogen content of the gaseous products is higher indicating that another pyrolysis is taking place.

Carbon disulphide, carbonyl sulphide, and thiophene are also formed in traces. The occurrence of carbon disulphide indicates that methane has reacted with sulphur vapor (Eq. [6]). This reaction is the basis of a commercial process to make carbon disulphide:

$$CH_4+4S \longrightarrow CS_2+2H_2S . \qquad [6]$$
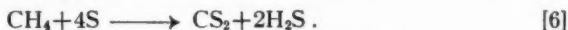
The presence of carbonyl sulphide is due to some oxygen in the system, probably some oxide impurity in the catalyst, and the presence of thiophene is due to the dehydrogenation of diethyl sulphide or to the condensation of ethylene with hydrogen sulphide followed by dehydrogenation.

REFERENCES

1. BARTUNEK, P. F. and BARKER, E. F.   Phys. Rev. 48: 516.   1935.
2. GODART, J.   J. chim. phys. 34: 70.   1937.
3. HERZBERG, G.   Infrared and Raman spectra of polyatomic molecules.   Vól. II.
   3rd printing.   D. Van Nostrand Company, Inc., New York.   1947.   pp. 271–369.
4. SABATIER, S. and MAILHE, C. R.   Compt. rend. 150: 1571.   1910.

GLYCERIDE SYNTHESES

II. PREPARATION OF SYMMETRICAL SATURATED MONOACID DIGLYCERIDES FROM 2-O-BENZYLGLYCEROL[1]

By A. J. E. Porck[2] and B. M. Craig

ABSTRACT

A direct preparation of symmetrical saturated monoacid diglycerides is described. The synthesis involves the preparation of 2-O-benzylglycerol, acetylation, interesterification with either methyl stearate or methyl palmitate, and hydrogenolysis to yield the desired 1,3-diglyceride. The melting points of the symmetrical diglycerides are slightly higher than those obtained previously by other methods of synthesis which involve a 1,2 to 1,3 shift of acyl groups.

A previous publication from this laboratory (1) dealt with methods for the synthesis of symmetrical diglycerides by interesterification between the methyl esters of long chain fatty acids and 1,2-O-diacetyl glycerides, wherein the third hydroxyl of the glycerol was blocked. The present paper is a continuation of this research and concerns a procedure for the direct preparation of 1,3-O-disaturated glycerides which avoids the 1,2 to 1,3 shift and the use of fatty acid chlorides.

The starting material, 1,3-O-benzylideneglycerol, was readily prepared in large quantities by the procedure outlined by Verkade and van Roon (3), and was converted to 1,3-O-benzylidene-2-O-benzylglycerol by reaction with benzyl chloride in the presence of potassium hydroxide. The benzylidene group was removed by acid hydrolysis and the resultant 2-O-benzylglycerol was acetylated in 91% yield. This product was interesterified with methyl stearate or methyl palmitate to the respective 1,3-O-diacyl-2-O-benzylglycerol in 85% yield. Hydrogenolysis at normal pressure and 50° C. over palladous oxide gave the respective 1,3-O-diglycerides in nearly quantitative yield and high purity. The melting points of the symmetrical diglycerides are slightly higher than those found for other preparations of these products in which a 1,2 to 1,3 shift occurred. The method is very useful for the preparation of large quantities of saturated diglycerides owing to ease of purification of intermediates and final products.

EXPERIMENTAL

All melting points were taken on a heating stage microscope.

1,3-O-Benzylideneglycerol

1,3-O-Benzylideneglycerol was prepared by the procedure outlined by Verkade and van Roon (3). Reagent grade benzaldehyde and glycerol were used without further purification or drying. Fifteen times the amounts of starting materials employed by Verkade and van Roon were heated in a vigorous stream of carbon dioxide at 170° C. for four hours. The solid product was

dissolved in chloroform instead of benzene. The same yield (60%) as reported by Verkade and van Roon was obtained. The 1,3-O-benzylideneglycerol was recrystallized from benzene – petroleum ether (Skellysolve "B") to a melting point of 79–82° C., reported m.p. 82.5–83.5° C. (3).

1,3-O-Benzylidene-2-O-benzylglycerol

The crude 1,3-O-benzylideneglycerol (250 gm.) was dissolved in 2 liters of benzyl chloride (reagent grade, distilled *in vacuo* and stored over potassium hydroxide pellets) by stirring with a high speed stirrer (Ultra-Turrax, Canadian Laboratory Supplies). Finely powdered potassium hydroxide (470 gm.) was added portionwise to the solution with continuous stirring and the temperature of the reaction mixture was maintained between 80° and 90° C. by cooling. After the addition was completed the stirring was continued at the same temperature for one hour with no precautions to exclude moisture. The reaction mixture was cooled to room temperature, washed three times with twice the volume of water, and dried over anhydrous potassium carbonate. The excess benzyl chloride was removed by distillation under 5 mm. pressure. The residue solidified on cooling and was dissolved in 440 ml. of hot benzene. One liter of hot petroleum ether (Skellysolve "B") was added to the solution and a precipitate appeared on cooling. The mixture was allowed to stand overnight in a refrigerator and was filtered, yield 294 gm. (78% of the theoretical amount), m.p. 76–78° C. Recrystallization from 220 ml. of benzene and 600 ml. of Skellysolve "B" gave 278 gm. of product, m.p. 77–78° C., reported m.p. 75.5–76.5° C. (4).

The product was also prepared by using an ordinary laboratory stirrer but the reaction time was extended to four to five hours at 100° C. and moisture was excluded from the reaction mixture. A similar yield was obtained but the crude product had a melting range of 70° to 75° C.

2-O-Benzylglycerol

The procedure of West and Ludwig (4) was modified slightly. Pure 1,3-O-benzylidene-2-O-benzylglycerol (250 gm.) was added to a solution of 80 ml. of concentrated sulphuric acid in 600 ml. of water and 400 ml. of ethanol. The mixture was refluxed for six hours, cooled to room temperature, steam distilled, and the residue extracted with ethyl ether. The ethereal solution was dried and the ether evaporated *in vacuo*, yield 178 gm. (96% of the theoretical amount). A product, m.p. 37–39° C. (reported m.p. 38.5–40° C. (4)), was obtained by recrystallization from benzene.

1,3-Di-O-acetyl-2-O-benzylglycerol

A mixture of 180 gm. 2-O-benzylglycerol, m.p. 37–39° C., 900 ml. acetic anhydride, and 60 gm. anhydrous sodium acetate was maintained at 100° C. for three hours with occasional shaking. The major portion of the excess acetic anhydride was removed by distillation under reduced pressure. The sodium acetate which precipitated caused bumping and was removed by cooling the mixture and filtering. The remainder of the acetic anhydride was removed by distillation under reduced pressure, and the residual oil was distilled *in vacuo*.

Yield of colorless liquid was 239 gm. (91% of the theoretical amount), b.p. 142° at 0.7 mm. Removal of excess acetic anhydride was also accomplished by heating with methanol and addition of ether. Sodium acetate, methyl acetate, and acetic acid were washed from the ethereal solution with water, 5–10% aqueous sodium carbonate, and water, successively.

A second distillation yielded a pure product, m.p. 14° C., n_D^{25} 1.4884. Calc. for $C_{14}H_{18}O_5$: C, 63.14%; H, 6.81%. Found: C, 63.36%; H, 6.91%. Acetylation with pyridine and acetic anhydride in the usual manner gave an equally satisfactory yield of the diacetate. 1,3-Di-O-acetyl-2-O-benzylglycerol is miscible with ethanol, ethyl ether, and benzene, slightly soluble in petroleum ether (Skellysolve "F"), and insoluble in water.

1,3-Di-O-stearoyl-2-O-benzylglycerol

A mixture of 20 gm. 1,3-di-O-acetyl-2-O-benzylglycerol (0.075 mole), 44.8 gm. methyl stearate (0.15 mole), and 1.2 ml. of 1.0 N sodium methoxide in methanol was heated at 100° C. on a rotary evaporator at 20 mm. Hg pressure for one hour. The reaction mixture was taken up in 400 ml. ethyl ether. The ethereal solution was washed twice with 500 ml. water, dried over anhydrous sodium sulphate, and the ether was evaporated under reduced pressure. The crude product was crystallized from 500 ml. acetone, yield 46 gm. (85% of the theoretical amount), m.p. 48.5° C. Further recrystallizations from acetone gave the analytically pure compound, m.p. 49.5° C. Calc. for $C_{46}H_{82}O_5$: C, 77.26%; H, 11.56%. Found: C, 77.36%; H, 11.54%.

The 1,3-di-O-stearoyl-2-O-benzylglycerol is soluble in acetone, petroleum ether (Skellysolve "F"), chloroform, benzene, and ethyl acetate, slightly soluble in methanol and ethanol, and insoluble in water.

1,3-Di-O-palmitoyl-2-O-benzylglycerol

This compound was prepared as described above for the stearoyl derivative in 85% yield. The crystallized product had m.p. 40° C. Calc. for $C_{42}H_{74}O_5$: C, 76.54%; H, 11.32%. Found: C, 76.52%; H, 11.39%.

1,3-Di-O-stearoylglycerol

A solution of pure 1,3-di-O-stearoyl-2-O-benzylglycerol (10 gm.) in 100 ml. of ethyl acetate (reagent grade) was hydrogenated for three hours at normal pressure and 50° C. with 200 mgm. palladous oxide. The catalyst was removed by filtration and the 1,3-di-O-stearoylglycerol was precipitated by cooling the solution in a dry ice – acetone bath, yield 8.6 gm. (98% of the theoretical amount), m.p. 80° C. (reported m.p. 79.5° C. (2)).

1,3-Di-O-palmitoylglycerol

This product was obtained in the same manner in 97% yield, m.p. 73.5° C., reported m.p. 72.5° C. (2).

REFERENCES

1. BARRY, P. J. S. and CRAIG, B. M.   Can. J. Chem. 33: 716.   1955.
2. JACKSON, F. L., DAUBERT, B. F., KING, C. G., and LONGENECKER, H. E.   J. Am. Chem.
    Soc. 66: 289.   1944.
3. VERKADE, P. E. and VAN ROON, J. D.   Rec. trav. chim. 61: 831.   1942.
4. WEST, W. A. and LUDWIG, B. J.   J. Am. Chem. Soc. 74: 4466.   1952.

THE PAPILIONACEOUS ALKALOIDS

XXI. THE ALKALOIDS OF *LUPINUS PILOSUS* WALT. AND THE STRUCTURE OF TETRALUPINE[1]

By A. F. Thomas,[2] H. J. Vipond,[2] and Léo Marion

ABSTRACT

The alkaloidal extract from *Lupinus pilosus* Walt. was found to contain two alkaloids, one widely occurring one, *d*-lupanine, and one which had already been reported to occur in this plant, i.e., *d-epi*lupinine, but no lupinine. The alkaloid tetralupine has been found by direct comparison of the bases and their corresponding salts and by their infrared absorption spectra to be identical with *dl-epi*lupinine. A study of the infrared absorption spectra of lupinine and *epi*-lupinine leads to the same conclusion as the chemical evidence, i.e., that in the former the hydroxymethylene group occupies the axial position whereas in *epi*lupinine this group is in the equatorial position.

Although *Lupinus pilosus* has been mentioned as a source of *l*-lupinine (2), White (11) reported *d-epi*lupinine as the only identifiable alkaloid in the plant. Since there was available a crude alkaloidal extract of *Lupinus pilosus* Walt. (*Lupinus villosus* Willd.) prepared and given to us by Dr. James F. Couch, the alkaloids were reinvestigated. The crude extract consisted of a thick dark syrup. A quantity of the syrup was dissolved in benzene, chromatographed on a column of alumina, and eluted with a number of solvents taken in the order benzene–ether–chloroform–methanol, the proportions of each being gradually changed. A trace of non-alkaloidal material was eluted first. Solvents containing benzene (40%) and ether (60%) to ether (95%) and chloroform (5%) eluted a colorless oil which behaved chromatographically on buffered paper as *d*-lupanine. This base formed a crystalline perchlorate, the melting point of which was undepressed on mixing with *d*-lupanine perchlorate. The identity of this salt was further confirmed by comparison of its Debye-Scherrer powder diagram with that of authentic *d*-lupanine perchlorate. This fraction composed about 15% of the crude mixture.

Fractions eluted in the solvent range ether (60%) – chloroform (40%) to ether (40%) – chloroform (60%) gave crystals, m.p. 76–78°, representing 35% of the original crude mixture. This crystalline substance on a paper chromatogram behaved like lupinine, but mixing with the latter lowered the melting point to 43–47°. The crystalline base, after recrystallization, melted at 78–79° and had $[\alpha]_D$ +37.1° (12), which agreed with the properties reported for *d-epi*lupinine, and the melting point was undepressed in admixture with a sample of the latter prepared as described by Schöpf, Schmidt, and Braun (10). The infrared absorption spectra of the two were superimposable (Fig. 1, curve 1) but different from that of lupinine (Fig. 1, curve 2).

Note added in proof: *After submission of the manuscript, a paper was published by W. D. Crow and N. V. Riggs (Australian J. Chem. 8: 136. 1955) reporting the occurrence of d-epilupinine and d-epilupinine-N-oxide in* Lupinus varius *L.*

In order to ascertain that *epi*lupinine did not arise on the column of alumina by epimerization of lupinine, some of the latter was chromatographed in the same way and eluted with ether containing increasing quantities (up to 10%) of chloroform. All the lupinine was recovered unchanged.

The remaining fractions from the main chromatogram yielded a dark brown glass which resisted all attempts at crystallization, and failed to give either a
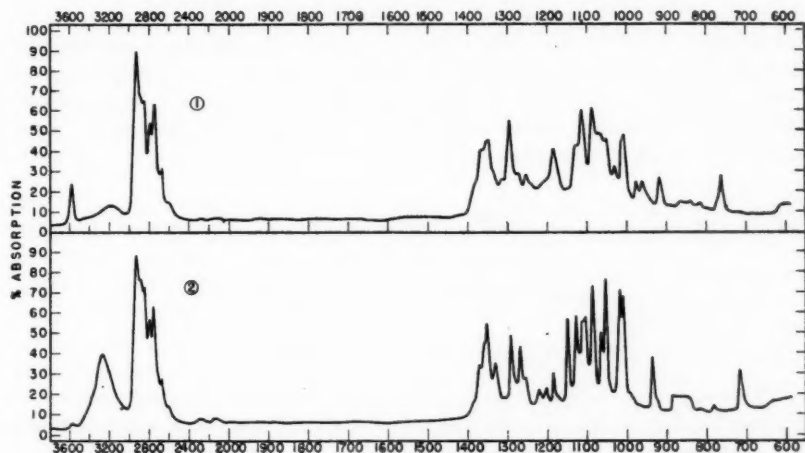
FIG. 1.   Infrared absorption spectra taken in carbon disulphide solution on a Perkin-Elmer double beam spectrophotometer, model 21. Curve 1, *epi*lupinine; curve 2, lupinine.

crystalline picrate or perchlorate. When this glass was chromatographed on paper, it gave a number of indeterminate streaks. A paper chromatogram of the original crude alkaloidal extract had shown that there were only two major components which are, therefore, *d*-lupanine and *d-epi*lupinine.

As previously recorded (10, 12) *d-epi*lupinine showed a depression in melting point when mixed with the isomeric alkaloid tetralupine discovered by Couch[1] in 1934 (4). A sample of tetralupine was dissolved in benzene, chromatographed on a column of alumina, eluted, sublimed *in vacuo*, and recrystallized from petroleum ether. It then melted at 83° and was optically inactive (Couch had reported $[\alpha]_D$ +4.63°). It formed a picrolonate, a picrate, and a methiodide which were all crystalline. Comparison of tetralupine and its salts by melting point and mixed melting point with authentic samples of *dl-epi*lupinine and its corresponding salts, kindly supplied by Professor G. R. Clemo, showed the identity of the two bases. The identity was confirmed by the infrared absorption spectrum of tetralupine which was exactly superimposable on those of *d-epi*lupinine and *dl-epi*lupinine. It can, therefore, be concluded unambiguously that tetralupine is identical with *dl-epi*lupinine.

An examination of the infrared absorption spectra of lupinine and *epi-*

[1]Dr. James F. Couch, about a year before his death, had most generously given to the senior author his entire supply of tetralupine.

lupinine in nujol mulls shows that both contain a broad bonded OH band at
ca. 3160 cm.$^{-1}$, but that in carbon disulphide solution the hydroxyl band in
the spectrum of *epi*lupinine becomes sharp and is shifted to 3580 cm.$^{-1}$,
while in the spectrum of lupinine the hydroxyl band moves only to 3270 cm.$^{-1}$
and remains broad.

This observation is indicative that in lupinine the hydroxyl group is hydro-
gen bonded, as previously inferred by Cookson (3), whereas it is not in *epi*-
lupinine. It is also significant that lupinine is eluted from an alumina column
before *epi*lupinine. Consequently, lupinine must be assigned a conformation
with the hydroxymethylene group occupying a position in which it may easily
form a hydrogen bond to the nitrogen atom. Such hydrogen bonding is possible
in the axial position I, but is impossible in the equatorial position II. Hence
formula I must be assigned to lupinine while formula II must represent
*epi*lupinine.

I
LUPININE

II
EPILUPININE
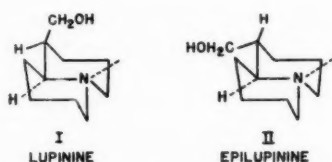
This assignment of conformations from the absorption characteristics of the
hydroxyl group in the infrared spectra of the two bases is in agreement with
the conformations assigned recently by Galinovsky and Nesvadba (6) to
lupinine and *epi*lupinine from the interpretation of the rearrangement readily
undergone by the tosyl ester of lupinine and the stability of the tosyl ester of
*epi*lupinine. Lupinine can be degraded to 2-*n*-butyl-3-methylpiperidine (12)
and by an unambiguous synthesis of the isomers of this substance Leonard
and Ryder (7) have correlated the stereochemistry of the degradation product
with that of lupinine. Recently, Ratuský, Reiser, and Šorm (9) by a study of
the dipole moments of lupinine and *epi*lupinine as well as of the two forms of
3-hydroxymethylquinolizidine have reached the same conclusion as to the
conformations of the two alkaloids. Consequently, all the evidence points to
formula I for lupinine and formula II for *epi*lupinine.

Paper chromatography has been shown to be a useful preliminary tool to
examine the constituents of a crude alkaloidal mixture in the lupine series.
When using sodium phosphate – citric acid buffers and saturated aqueous
n-butanol as the developing agent, the R_F values obtained for a group of
lupine alkaloids show that they can be separated easily. The spots were detected
with the Munier-Dragendorff reagent (8). The papers were buffered by dipping
in a buffer solution of appropriate strength, blotting, and allowing to dry
completely (contrast Brindle *et al.* (1)).

EXPERIMENTAL

d-Lupanine

A quantity (1.0 gm.) of the crude alkaloidal extract was dissolved in dry
benzene (100 ml.), the solution filtered, and chromatographed on alumina.

The column was eluted with benzene, ether, chloroform, and methanol, 98 fractions of 60 ml. each being collected. The initial fractions contained non-basic material. From fractions 26–43 (60% ether – 40% benzene to 95% ether – 5% chloroform) a colorless oil was obtained (150 mgm.) which with 60% perchloric acid gave a perchlorate $[\alpha]_D^{19}$ +46° (c, 1.0 in water), m.p. 207–210°, and after two recrystallizations from methanol, m.p. 211°, either alone or in admixture with an authentic sample of d-lupanine perchlorate. The literature (5) gives $[\alpha]_D$ +46.8°. The identity of the perchlorate was further confirmed by comparison of its Debye-Scherrer powder diagram with that of authentic d-lupanine perchlorate. Each fraction had been shown by paper chromatography at pH 7 to be the same, and to behave in the same way as d-lupanine with varying pH.

d-Epilupinine

Fractions 53 (60% ether – 40% chloroform) to 70 (40% ether – 60% chloroform) of the chromatogram yielded a crystalline substance, 350 mgm., m.p. 76–78°, which after two recrystallizations from petroleum ether melted at 78–79° and had $[\alpha]_D^{19}$ +37.1° (c, 1.0 in ethanol). Found: C, 71.4; H, 11.1. Calc. for $C_{10}H_{19}ON$: C, 71.0; H, 11.3%. The melting point of this base was unaffected by mixture with d-epilupinine, but depressed to 68–78° by admixture with tetralupine, and to 43–47° by admixture with lupinine. Comparison of the infrared absorption spectra confirmed the identity of the base with d-epilupinine.

Chromatography of Lupinine

Lupinine (390 mgm.) was dissolved in ether and chromatographed on alumina. It was eluted at once by the same solvent, the last traces being removed with ether – 10% chloroform.

Tetralupine

A quantity of tetralupine was chromatographed on alumina and eluted exactly as described for d-epilupinine. The substance was sublimed in a high vacuum and recrystallized from petroleum ether from which it separated in small colorless prisms, m.p. 83°, and in admixture with an authentic sample of dl-epilupinine, m.p. 82–83°, $[\alpha]_D$ 0 in ethanol. The infrared absorption spectrum of tetralupine was superimposable on that of dl-epilupinine. Tetralupine formed a picrolonate, m.p. 231–232°, undepressed by admixture with dl-epilupinine picrolonate (m.p. 225°). Found: C, 55.41; H, 5.97. Calc. for $C_{10}H_{19}ON \cdot C_{10}H_{27}O_6N_5$: C, 55.41; H, 6.28%. The picrate consisted of yellow needles, m.p. 145.5–147°, either alone or in admixture with synthetic dl-epilupinine picrate. Found: C, 48.18, 48.41; H, 6.09, 5.46. Calc. for $C_{10}H_{19}ON \cdot C_6H_3O_7N_3$: C, 48.24; H, 5.57%. Tetralupine also formed a methiodide, m.p. 251–253°, either alone or in admixture with dl-epilupinine methiodide (m.p. 248°). Found: C, 42.92; H, 7.31. Calc. for $C_{11}H_{22}ONI$: C, 42.44; H, 7.13%.

*epi*lupinine and its salts, and to Mr. R. Lauzon and Dr. R. N. Jones of these laboratories for taking the infrared absorption spectra.

REFERENCES

1. BRINDLE, H., CARLESS, J. E., AND WOODHEAD, H. B.   J. Pharm. and Pharmacol. 3: 793. 1951.
2. CLEMO, G. R. and RAPER, R.   J. Chem. Soc. 1927. 1929.
3. COOKSON, R. C.   Chemistry & Industry, 337. 1953.
4. COUCH, J. F.   J. Am. Chem. Soc. 56: 2434. 1934.
5. EDWARDS, O. E., CLARKE, F. H., and DOUGLAS, B.   Can. J. Chem. 32: 235. 1954.
6. GALINOVSKY, F. and NESVADBA, H.   Monatsh. 85: 1300. 1954.
7. LEONARD, N. J. and RYDER, B. L.   J. Org. Chem. 18: 598. 1953.
8. MUNIER, R.   Bull. soc. chim. biol. 35: 1225. 1953.

9. RATUSKÝ, J., REISER, A., and ŠORM, F.   Chem. Listy, 48: 1795. 1954.
10. SCHÖPF, C., SCHMIDT, E., and BRAUN, W.   Ber. 64: 683. 1931.
11. WHITE, E. P.   New Zealand J. Sci. Technol. 33: 50. 1951.
12. WINTERFELD, K. and HOLSCHNEIDER, F. W.   Ber. 64: 137. 1931.

THE SELECTIVE DEGRADATION OF WHEAT GLUTEN[1]

By L. Wiseblatt,[2] L. Wilson,[3] and W. B. McConnell

ABSTRACT

A method believed to hydrolyze peptide bonds of proteins selectively at the amino groups of serine was used to obtain polypeptides from wheat gluten. The procedure involved the use of strong acid and introduced appreciable amounts of sulphur into the products possibly as sulphonic acid groups. Most of the serine appeared at the amino termini of the peptides. The peptides displayed a striking electrophoretic homogeneity which may at least in part be accounted for by the acquired acid groups. Osmotic pressure measurements indicated an average molecular weight near 20,000 and terminal group estimates indicate that each molecule contained several N-terminal serine residues. There appeared to be strong association or chemical cross linking between peptide chains of the degraded gluten.

INTRODUCTION

In 1948 Desnuelle and Casal (8) showed that when proteins are hydrolyzed in 10 N hydrochloric acid at 30°C. the amino groups of the hydroxy amino acids, serine and threonine, are released much more rapidly than those of other amino acids. They suggested that the increased rate of rupture of these bonds involved a preliminary migration of the acyl part of the peptide bond from the amino group to the hydroxyl group of the hydroxy amino acid. The mechanism probably involves formation of an intermediate hydroxyoxazolidine structure as follows (1, 9):
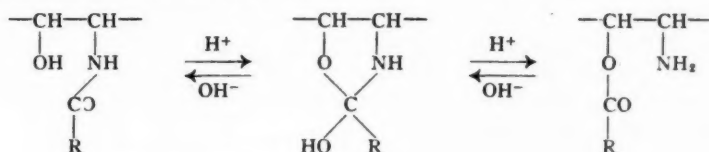
More recently Desnuelle and Bonjour (7) observed that a more specific hydrolysis at hydroxy amino acids could be obtained by pretreating the protein with cold anhydrous sulphuric acid according to the method of Reitz, Ferrel, Fraenkel-Conrat, and Olcott (19). Subsequent hydrolysis in 6 N hydrochloric acid at 18°C. released the amino groups of the hydroxy amino acids many times more rapidly than those of all other amino acids.

Elliott (9) utilized the above observations in an ingenious manner. Silk fibroin was treated with cold concentrated sulphuric acid to produce the N-peptidyl → O-peptidyl shift. The product was then acetylated to block the newly freed amino groups. Subsequent treatment with cold dilute barium hydroxide saponified the ester links yielding N-acetyl peptides. About 60%

of the serine and no threonine was N-terminal. The absence of terminal threonine was not considered very significant because of the very low percentage of that amino acid in silk. In a subsequent degradation of lysozyme Elliott (11) found that nearly all the serine and about one-third of the threonine was terminal. The amino groups of serine and threonine accounted for all of the amino nitrogen in the degradation products of lysozyme (11) whereas a small amount of non-specific hydrolysis of silk fibroin occurred.

This communication is a report of results obtained when Elliott's degradation scheme was applied to wheat gluten. The method seemed particularly attractive because serious solubility problems are encountered in almost all chemical investigations of native gluten. Thus denaturation occurs irreversibly in dilute alkali, the only aqueous medium which gives apparently monodisperse solutions, and aqueous acetic acid, sodium salicylate, and urea do not give molecular dispersions of the protein. If gluten could be specifically degraded to soluble products, these difficulties would be partly overcome. Information from a study of degradation products might well help elucidate the constitution of gluten.

EXPERIMENTAL METHODS AND RESULTS

Sulphuric Acid Treatment of Gluten

Ten grams of wheat gluten prepared by the method of Lusena (15) was suspended in 200 ml. of anhydrous sulphuric acid (sulphuric acid containing 10% by volume of 20% oleum). After 24 hr. at $-20°C$. the mixture was allowed to warm slowly to room temperature and was left to stand with occasional shaking for another five days. The resulting viscous brown liquid was poured with vigorous stirring into 2 liters of anhydrous ether at $-35°C$. The precipitate was collected by centrifugation, washed three times with dry ether, and finally added to 600 gm. of crushed ice on which it formed a sticky yellow mass.

Degradation of Gluten Sulphate

The suspension obtained above was adjusted to pH 5 with solid sodium acetate and treated over a period of two hours with 100 ml. of acetic anhydride. The temperature was not allowed to exceed 5°C. Sodium acetate was added as required to maintain pH at 5. The reaction products were dialyzed against water until they reached a constant low acidity as measured by titration or until the washings were acetate free as indicated by the sensitive lanthanum nitrate spot test (7, 13). The dialysis washings were discarded after they had been shown to be nitrogen free. The contents of the dialysis bag were reduced to about 100 ml. in a stream of air and the gluten derivative, which settled out as a spongy mass, was collected and added to 100 ml. of 0.1 N barium hydroxide. After two hours at room temperature all material dissolved to yield a yellowish brown solution. This was saturated with carbon dioxide and the precipitated barium carbonate discarded after it had been shown to be free of nitrogen. The supernatant solution was freeze dried to yield about 9 gm. of acetyl peptide.

In an attempt to remove acetyl groups 1 gm. of acetyl peptide was shaken with a mixture of 100 ml. of dry methanol and 1 ml. of concentrated hydro-

chloric acid for three days at 5°C. (In a few later experiments a 1% solution of anhydrous hydrochloric acid in dry methanol was used to reduce unspecific peptide bond hydrolysis.) The solid residue was filtered off, washed with methanol, then dissolved in water and freeze dried yielding a pale brown methanol insoluble peptide (hereinafter called MIP). The filtrate was evaporated at room temperature *in vacuo*, the residue taken up in water and freeze dried to yield the methanol soluble peptide (MSP). Both MSP and MIP were readily soluble in water, yielding solutions which foamed readily and which gave precipitates with trichloroacetic, tannic, and metaphosphoric acids.

MSP gave a strong color reaction with ninhydrin but both the original acetyl peptide and MIP were ninhydrin negative. It appeared therefore that deacetylation was incomplete and that only MSP contained free amino groups. This suggestion was supported by the qualitative detection of acetyl groups in MIP but not in MSP. A modification of the method of Clark (6) was used for acetyl determinations but as might be expected with N-acetyl derivatives of comparatively long chain peptides quantitatively reliable data could not be obtained. It was further observed that MIP did not contain amino groups detectable by either the formal titration or the Van Slyke nitrous acid method of deamination.

Reitz *et al*, (19) have shown that the preferential reaction of sulphuric acid with proteins is the formation of sulphuric esters with aliphatic hydroxyl groups of hydroxy amino acids followed by sulphonation of the aromatic ring of tyrosine and conversion of cystine and cysteine to thiosulphates. Some sulphur determinations were, therefore, done on the degradation products by the method of Sundberg and Royer (21). All analyses showed that sulphuric acid treated gluten contained about three times as much sulphur as the original gluten. For example, a preparation of acetyl peptide contained 3.5% sulphur whereas the gluten from which it was derived contained 1.1% sulphur. This is approximately equal to the addition of one mole of sulphur for each 12–14 amino acid residues. This amount would be expected to contribute in an appreciable way to the properties of the peptides.

Terminal Group Determinations

Sanger's method (20) of detecting N-terminal amino acids by reaction with 1-fluoro-2,4-dinitrobenzene (DNFB) was applied to the methanol soluble peptides. Seventy-five milligrams MSP were dissolved in 5 ml. of water, and 100 ml. of sodium bicarbonate and 250 mgm. of DNFB in 10 ml. of ethanol were added. The mixture was shaken for three hours, evaporated to dryness, and extracted with ether until no more of the yellow reagent could be removed. The residue was hydrolyzed by boiling in 6 N hydrochloric acid for eight hours and after evaporating to dryness *in vacuo* it was extracted with ether to dissolve any dinitrophenyl (DNP) amino acids produced from N-terminal amino acids.

The ether extracts were chromatographed on No. 1 Whatman filter paper impregnated with pH 6 phthalate buffer and were developed by both ascending and descending flow. The following solvent systems were used: 30 : 70

propanol–cyclohexane; 10 : 90 ethanol – benzyl alcohol and tertiary amyl alcohol (2). DNP acids for comparison were prepared as described by Porter (18). Except for spots due to the reagent and to 2,4-dinitroaniline the chief spot on all chromatograms corresponded to DNP serine. The yellow spots were extracted from the paper with ether and hydrolyzed in a sealed tube at 100°C. for two hours with concentrated aqueous ammonia. When these hydrolyzates were chromatographed on paper using 75% aqueous phenol as solvent they all gave ninhydrin positive spots with the same R_f value as serine.

The above results strongly suggest that serine is a major N-terminal amino acid residue in degraded gluten. Quantitative estimates of N-terminal serine and threonine in MSP were attempted by a microdiffusion method employing periodate oxidation (23). No N-terminal threonine was found but ammonia corresponding to 5.7 gm. serine per 16 gm. MSP nitrogen was obtained. Since Wiseblatt and McConnell (23) found by the same method that gluten contains 5.7 gm. serine per 16 gm. gluten nitrogen, the result indicates complete liberation of serine amino groups by the Elliott degradation. The value for terminal serine in degraded gluten is high compared to Elliott's yields of about 60% for silk fibroin. An experimentally reproducible value for N-terminal serine (7.7 gm. serine per 16 gm. nitrogen) was obtained with a colorimetric method in which formaldehyde from periodate oxidation was collected by distillation (4). Since carbohydrates, some of which could also have given formaldehyde, were shown (17) to be present only in trace amounts no explanation for the high results can be offered. Although the results may not be quantitatively acceptable they do support the qualitative suggestion that the degradation scheme used was effective in liberating much of the serine nitrogen in gluten.

Preliminary experiments were attempted to identify C-terminal amino acids present in the peptides. Because appreciable N-terminal serine appeared to be liberated the C-terminal residues may be assumed to be largely those bound to serine in the original gluten. Samples of both the MIP and MSP were refluxed for eight hours with equal weights of lithium aluminum hydride in 100 volumes of tetrahydrofuran. The products were hydrolyzed and the amino alcohols isolated by the methods of Fromageot, Jutisz, Meyer, and Penasse (12). Paper chromatography of the ether extract of each hydrolyzate with the recommended solvent systems gave mainly one ninhydrin positive spot. (R_f corresponded most closely to that of phenylalaninol.) Other spots were present in smaller quantities. It is emphasized that the results are not sufficient to show that there is one major C-terminal amino acid, but they suggest that there may be a relatively simple pattern of C-terminal residues. More experiments are required on this phase of the work before definite statements regarding the liberation of C-terminal amino acids can be made.

Electrophoretic Properties

The apparatus and procedure described by Kunkel and Tiselius (14) was used for paper electrophoresis of some products of the gluten degradation. Fig. 1 shows "ionograms" obtained from acetyl peptides, MIP, and MSP in acetate buffer at pH 4.5 and ionic strength 0.1. With each sample a single,

fairly well defined peak was obtained. There was no indication of a multiplicity of peaks or of marked spreading as ordinarily obtained with a heterogeneous substance. Paper ionograms were made with a number of different preparations of the gluten degradation products and it is significant that single peaks were obtained (Fig. 1). The results served to distinguish sharply between the MSP
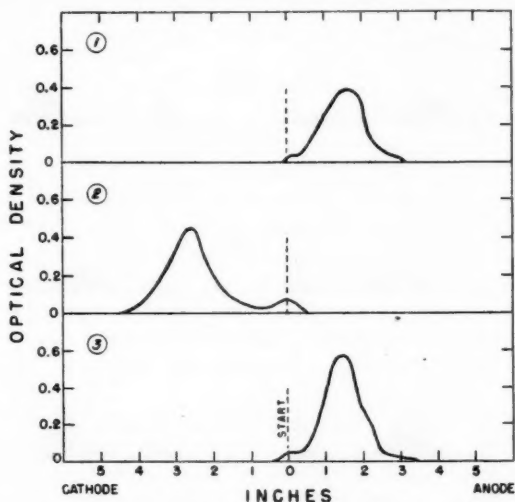
FIG. 1.   Results of filter paper electrophoresis on gluten degradation products.
Acetate buffer   pH 4.5   ionic strength 0.1
1. Acetyl peptides   22 hr.   0.25 ma. per cm.
2. MSP   28 hr.   0.20 ma. per cm.
3. MIP   28 hr.   0.20 ma. per cm.

and MIP as derived from the deacetylation step and are in general accord with the earlier suggestion that the latter was not deacetylated.

A limited number of experiments were made with a moving boundary electrophoresis apparatus (Aminco Portable, American Instrument Co., Silver Springs, Maryland). MSP and MIP which had been kept in 0.1 N NaOH at 5°C. for 16 hr., freeze dried, taken up in the desired buffer, and filtered were used. Drawings of the patterns obtained with the treated MSP are given in Fig. 2. The alkali treatment had evidently changed the electrophoretic properties of MSP but, as with experiments on paper, the method failed to effect any separation of the material into fractions of different mobility. On the contrary, the sample displayed a striking electrophoretic homogeneity, and in buffers of pH 8.6, 6.0, and 4.5 it had mobilities of 2.08 $\times$ 10^{-4}, 1.91$\times$10^{-4}, and 1.31 $\times$ 10^{-4} cm.^{2}/sec./volt, respectively. If acidic groups had been introduced perhaps during the sulphuric acid treatment, the movement toward the anode would have been expected. The MIP treated with sodium hydroxide as described above gave patterns of the same general character as shown in Fig. 2 for sodium hydroxide treated MSP.

The reason for the markedly different behavior of MSP before and after alkali treatment is not known. It is likely that many of the acidic groups on the dissolved material were converted to methyl esters during the treatment with methanolic hydrochloric acid and that before saponification at 5°C. deacetylated amino groups caused migration toward the cathode (Fig. 1). It would appear, therefore, that in the saponified peptides a preponderance of
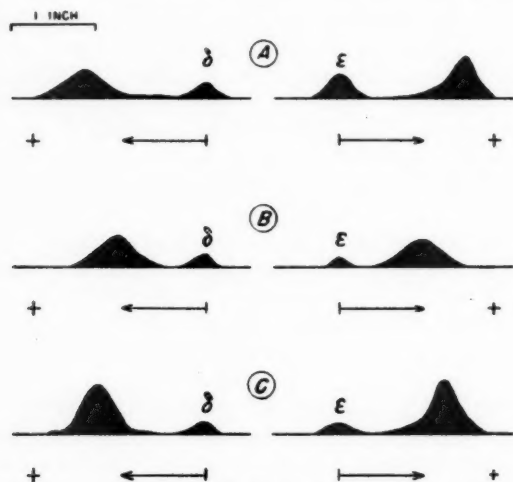
FIG. 2.   Electrophoretic patterns of MSP obtained with Aminco Portable Electrophoresis Apparatus.

A. Barbitol buffer	pH 8.6	ionic strength 0.1	3130 sec. at 5.7 volts/cm.
B. Cacodylate buffer	pH 6	ionic strength 0.1	5680 sec. at 2.3 volts/cm.
C. Acetate buffer	pH 4.5	ionic strength 0.1	7850 sec. at 3.2 volts/cm.

acidic groups was effective in masking charge differences arising from different degrees of deacetylation of amino groups. If, as is reasonable to suppose, the sulphur acquired by the peptides during the degradation occurs as ionizable acid groups, and if, after treatment with sodium hydroxide the acid groups are free, the peptides would be expected to behave as anions. The contribution of the acidic groups to electrical properties of the material might well be large compared to that from amino groups and may account for the observed electrophoretic homogeneity.

By contrast, gluten is known to be heterogeneous according to physical chemical criteria. It seems unlikely, therefore, that the degradation is sufficiently systematic and the array of amino acids in gluten sufficiently regular to yield peptides with the degree of homogeneity suggested by the electrophoretic results. Because the contribution of sulphonic acid groups to the electrical properties is not known it is difficult to determine whether the degradation product is more or less homogeneous than gluten.

The introduction of acid groups into the degradation products may also

explain results obtained in attempts to fractionate them by selective adsorption on ion exchange resins. Samples were applied to 100 cm. columns of either 12% or 2% cross linked Dowex 50 and eluted according to procedures described by Moore and Stein (16). The bulk of the peptide nitrogen was quickly eluted from the column in pH 3.5 buffer. No other nitrogenous material was detected by nesslerization in eluates obtained with buffers of increasing pH.

Molecular Size Estimates

From the value of 5.7 gm. N-terminal serine per 16 gm. of MSP nitrogen given above it was calculated that the average molecular weight of MSP would be not more than 1400 (assuming single unbranched chains). Such a peptide would have been expected to pass freely through a dialyzing membrane. Experiments were therefore done to determine directly the molecular weight of MSP in pH 4.5 acetate buffer of ionic strength 0.1. Osmotic pressure measurements were made according to the method of Bull (5) except that semi-permeable membranes of cellophane transfusion tubing were used instead of collodion bags. The average molecular weight was estimated to be 20,500. The value, although much larger than originally indicated by terminal amino acid analysis, was consistent with the physical behavior of MSP solutions upon dialysis.

The osmotic pressure equilibrium tended to drift slowly downward over a period of several days but the change did not represent a large increase in the molecular weight of the peptide. Redetermination of the terminal serine residues on MSP dialyzed in cellophane bags showed that, although appreciable material had passed through the membrane, the weight equivalent to each remaining terminal residue was not more than 3000. The results could be most readily explained by assuming that the degradation products consisted of branched chain structures with an average molecular weight of about 20,000. The number of free serine amino groups suggested that there are several chains in each molecule but the data were not considered sufficiently precise for a quantitative statement of the degree of branching.

Although MSP may possess branched chains it cannot also be assumed that undegraded gluten also possesses branched chains. For example, Elliott (11) found that lysozyme, which diffuses at an appreciable rate through a dialysis membrane, became non-diffusible after treatment with sulphuric acid and formic – acetic anhydride. A high proportion of the nitrogen was non-diffusible after alkaline hydrolysis. Elliott suggested that, since indoles polymerize under the influence of acid, cross linkages may have been formed through tryptophan residues and supported his suggestion with the observation that tryptophan could not be detected in the preparation by the Ehrlich color reaction. Although gluten probably contains no more than 1.5% tryptophan (22) a similar phenomenon may occur in its degradation.

Ultracentrifuge studies at 250,000 gravities for a period of 80 min. at 27°C. showed that the peptides were of "low" molecular weight—the "peak" had not completely separated from the meniscus after an hour. Also there was

sufficient broadening or spreading of the peak to indicate considerable hetero-
geneity and to render attempts at quantitative determination useless.

Formylation of Gluten Sulphate

One of the objectives of this study was to derive from gluten a uniform water
soluble product suitable for further study. Incomplete deacetylation with
resultant division of the product into MIP and MSP was therefore undesirable.

No other method for removal of blocking acetyl groups was known which
would not also lead to fission of peptide chains. Experiments were done to test
an alternative procedure for protecting amino acids exposed by the N-
peptidyl → O-peptidyl shift. Carbobenzoxylation seemed attractive except
that a relatively high sulphur content in the peptides might interfere with
catalytic hydrogenolysis. Recourse was taken therefore to a formylation
method suggested by Elliott (10). Boissonnas and Preitner (3) have demon-
strated that this group can be removed with methanolic hydrochloric acid
without hydrolysis of peptide chains. In this investigation it was observed that
ninhydrin positive groups of sulphuric acid treated gluten were completely
"formylated" by treatment at pH 5 with sodium formate and anhydrous
formic acid for 12 hr. at room temperature. As with acetylated materials
degradation to soluble products occurred upon saponification with 0.1 N
$Ba(OH)_2$. Treatment with 1.5 M hydrogen chloride in methanol at 20°C. for
24 hr. gave a 90–95% yield of a ninhydrin positive material soluble in metha-
nol. A comparison of the material with MSP has not been made but formyla-
tion appears superior to acetylation in experiments where recovery of peptides
with unprotected terminal amino groups is required. Work to be described in
a later publication suggests that deamination with nitrous acid can be used to
prevent reversal of the acyl migration from peptide nitrogen to β-hydroxy
group. Although the terminal amino acid is destroyed the method may some-
times be used to replace the blocking methods.

In the present work it was hoped to obtain a soluble derivative suitable for
further chemical studies on the nature of gluten. In general the results give
promise of meeting these objectives. Water soluble products were obtained
apparently with a high degree of specificity. These should be amenable to
study with experimental techniques not formerly applicable to gluten itself.
The indication, for example, of a fairly simple pattern of C-terminal amino
acid residue suggests that gluten may possess systematic structural features.
This is particularly true because although gluten contains about 25% glu-
tamic acid there was no evidence of appreciable amounts of C-terminal glu-
tamic acid in the peptides. Changes may occur however which limit the use-
fulness of the peptides for further study. An unknown amount of cross linking
of peptide chains may have occurred. If this is so it is impossible to estimate
the degree of cross linking in gluten from the amount of chain branching ob-
served in the degraded fractions. Furthermore, introduction of sulphonic acid
groups may markedly alter the electrical properties of the material. The
striking electrophoretic homogeneity of the peptides and the difficulty in
fractionating them on a Dowex column may have been caused by this modi-
fication.

ACKNOWLEDGMENT

REFERENCES

1. BERGMANN, M., BRAND, E., and WEINMANN, F. Hoppe-Seyler's Z. physiol. Chem. 131: 1. 1923.
2. BLACKBURN, S. and LOWTHER, A. G. Biochem. J. 48: 126. 1951.
3. BOISSONNAS, R. A. and PREITNER, G. Helv. Chim. Acta, 36: 875. 1953.
4. BOYD, M. J. and LOGAN, M. A. J. Biol. Chem. 146: 279. 1942. See also BLOCK, R. J. and BOLLING, D. The amino acid composition of proteins and foods. 2nd ed. Charles C. Thomas, Publisher, Springfield, Ill. 1951.
5. BULL, H. B. and CURRIE, B. T. J. Am. Chem. Soc. 68: 742. 1946.
6. CLARK, E. P. Ind. Eng. Chem. Anal. Ed. 8: 487. 1936; 9: 539. 1937.
7. DESNUELLE, P. and BONJOUR, G. Biochim. et Biophys. Acta, 7: 451. 1951.
8. DESNUELLE, P and CASAL, A. Biochim. et Biophys. Acta, 2: 64. 1948.
9. ELLIOTT, D. F. Biochem. J. 50: 542. 1952.
10. ELLIOTT, D. F. Chemistry & Industry, 86. 1952.
11. ELLIOTT, D. F. In A Ciba foundation symposium. The chemical structure of proteins. Edited by G. E. W. Wolstenholme and M. P. Cameron. J. & A. Churchill, Ltd., London, England. 1953.
12. FROMAGEOT, C., JUTISZ, M., MEYER, D., and PENASSE, L. Biochim. et Biophys. Acta, 6: 283. 1950.
13. KRÜGER, D. and TSCHIRCH, E. Ber. 62: 2776. 1929; 63: 826. 1930.
14. KUNKEL, H. G. and TISELIUS, A. J. Gen. Physiol. 35: 89. 1951.
15. LUSENA, C. V. Cereal Chem. 27: 167. 1950.
16. MOORE, S. and STEIN, W. H. J. Biol. Chem. 192: 663. 1951.
17. MORRIS, D. L. Science, 107: 254. 1948.
18. PORTER, R. R. Methods in medical research. Vol. 3. Edited by R. W. Gerard. Year Book Publishers, Inc., Chicago, Ill. 1950.
19. REITZ, H. C., FERREL, R. E., FRAENKEL-CONRAT, H., and OLCOTT, H. S. J. Am. Chem. Soc. 68: 1024. 1946.
20. SANGER, F. Biochem. J. 39: 507. 1945.
21. SUNDBERG, O. E. and ROYER, G. L. Ind. Eng. Chem. Anal. Ed. 18: 719. 1946.
22. WISEBLATT, L. Ph.D. Thesis, University of Saskatchewan, Saskatoon, Sask. October, 1952.
23. WISEBLATT, L. and McCONNELL, W. B. Can. J. Chem. In press.

THE PHOTOLYSIS OF ACETONE IN THE LIQUID PHASE: THE GASEOUS PRODUCTS[1]

By R. Pieck[2] and E. W. R. Steacie

ABSTRACT

An investigation has been made of the photolysis of liquid acetone in the temperature range from 55° to −25°C. The quantum yields of all products are small, and decrease strongly with decreasing temperature. It is concluded that the low yields can be explained both on the basis of the 'cage effect', and by the deactivation of an excited molecule. At high temperatures and intensities the gaseous products can be accounted for on the assumption that radicals have escaped from the 'cage' and react analogously to the gas-phase mechanism. At low temperatures ethane formation in the 'cage' may be of importance.

INTRODUCTION

The photolysis of acetone in the gas phase has been extensively investigated. The photolysis in the liquid phase was investigated by Bowen and co-workers (1, 2, 3). They found practically no gaseous products with acetone alone, while tertiary alcohols were produced when they worked in hydrocarbon solvents. Frankenburg and Noyes (4) have also investigated the photolysis of the liquid systems acetone–oxygen and acetone–heptane–oxygen. Their work with pure acetone was confined to a rough determination of quantum yields.

It seemed of interest to investigate the photolysis of pure liquid acetone to obtain information about the behavior of radicals in the liquid phase, and to see how far the mechanism parallels that in the gas phase.

EXPERIMENTAL

Materials

Acetone (Merck) was dried over potassium carbonate and purified by a bulb-to-bulb distillation. Deutero-acetone was prepared for us by Dr. L. C. Leitch of these laboratories. n-Heptane was a "Phillips Pure Hydrocarbon" product. Special care was taken in degassing these reagents, in view of the sensitivity of the reaction to traces of oxygen.

Apparatus

The acetone was irradiated in a quartz cell of approximately 5 cm. diameter and 0.05 cm. thickness. The cell was provided with two outlets, one of which was sealed off after filling, while the other, closed by a break-seal, could be connected to the analytical system. Between experiments the cell was washed with acetone, attached to the filling system, heated *in vacuo* at 200°C. for two hours, and filled by distilling acetone into it.

Irradiation was carried out in a thermostat consisting of a brass cylinder with double walls, the space between the walls being evacuated. The cell was placed in an aluminum block which was located in the thermostat in such a

way that the cell was as close as possible to the innermost of two quartz windows through which the light beam entered the thermostat. The thermostat was filled with ethyl alcohol purified by the method of Leighton (7).

For experiments above room temperature the thermostat was heated electrically by an immersion heater. For low temperature experiments a copper coil was immersed in the alcohol. The coil was connected to a large container of alcohol which was cooled by a small refrigeration unit and kept automatically at the desired temperature. The temperature could be maintained constant to within ±0.5°C.

Light Source

For most experiments the light source was a B.T.H. high pressure mercury lamp (type ME/D, 250 watts) operated on 220 v. d-c. The beam was roughly collimated by a quartz lens. A Corning filter (9-53) cut off wave lengths below 2800 Å. The beam thus consisted mainly of wave lengths around 3130 Å.

For experiments with light in the neighborhood of 2537 Å a Hanovia S500 lamp was used, together with a filter consisting of a quartz cell of 1 cm. thickness filled with a solution of diphenylbutadiene in ether (5).

The light intensity was reduced in some experiments by the use of quartz neutral density filters.

Analysis

A comparison of the infrared spectrum of pure acetone and that of a sample of irradiated acetone did not reveal the presence of any liquid products. However, in view of the large amount of acetone present, it is not surprising that small quantities of liquid products could not be detected. At temperatures below 0°C., however, a very small amount of liquid residue remained in the cell after acetone had been distilled off.

After an experiment the contents of the cell were distilled into a column, at the top of which was a "cold finger" maintained at −78°C. The acetone was refluxed continuously in the column to remove dissolved gases. The gaseous products were then fractionated by means of two modified Ward stills (8). The $CO-CH_4$ fraction was removed at −195°C., and the C_2H_6 fraction at −170°C. The amount of CO was determined by combustion of the $CO-CH_4$ fraction over hot copper oxide. In a number of cases check analyses were made with a mass spectrometer.

RESULTS

(A) Determination of the CO Quantum Yield

An estimate of the quantum yield of CO was made by comparing the photolysis in the liquid phase at 25°C. with the gas-phase photolysis at 164°C. A quantum yield of unity was assumed for the latter. Values obtained were:

$$\lambda > 2800 \text{ Å (mainly 3130 Å)} \quad 0.9 \times 10^{-4}$$
$$\lambda \sim 2537 \text{ Å} \quad 2.3 \times 10^{-4}.$$

The results are uncertain by a factor of about 2, and hence there is no certain

difference between the two wave lengths. The values are in agreement with
the rough estimate of Frankenburg and Noyes (4).

(B) Variation of the Rate with Intensity

Table I gives the results of experiments at four different temperatures and
at various light intensities. In view of the inhomogeneous nature of the light
absorption, too much accuracy cannot be expected as far as kinetic constants
are concerned, although the very short path length minimizes this effect.
It is evident from the table that, although there is a decided drift in some of
the constants, all products are formed at a rate proportional to the light
intensity to within a factor of 3.

TABLE I

VARIATION OF RATE WITH INTENSITY

Temp., °C.	Time, min.	Relative light int.	Products, molecules/cc./sec. $\times 10^{-13}$			$\dfrac{R_{CH_4}}{R_{C_2H_6}^{\frac{1}{2}}} \times 10^{-7}$	$\dfrac{CH_4}{C_2H_6}$
			CO	CH$_4$	C$_2$H$_6$		
55	100	1.00	20.7	565	9.5	57.9	59.5
55	155	0.50	18.5	298	3.1	53.5	96
55	300	0.36	12.2	246	3.5	41.6	73
55	752	0.16	6.25	125	2.16	26.8	58
40	120	1.00	16.3	360	5.5	48.5	65
40.5	100	1.00	14.6	340	4.2	52.4	81
41	100	1.00	18.2	387	5.1	54.1	76
40	230	0.50	11.75	244.0	2.70	47.0	90
40	260	0.20	3.7	63.6	0.42	31.0	151
40	305	0.20	4.1	84.1	0.78	30.1	108
40	1140	0.14	2.08	51.7	0.74	19.0	70
40	1050	0.14	2.1	50.2	0.62	20.2	81
40	1020	0.05	0.66	8.6	0.15	7.1	57
40	1410	0.02	0.54	4.2	0.13	3.7	32
31	210	1.00	13.2	213	3.5	36.1	61
31	270	0.36	5.8	135	1.95	30.6	69
31	315	0.20	3.6	64	0.64	25.3	100
31	850	0.14	2.3	43	0.35	23.0	123
31	952	0.05	0.73	11.8	0.15	9.7	79
31	1650	0.02	0.44	6.5	0.10	6.5	65
15	245	1.00	6.41	84.0	2.1	18.3	40
15	350	0.14	1.86	31.0	0.37	16.1	84
15	630	0.05	0.34	5.2	0.15	4.2	35

The last two columns give the ratios $R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}}$ and $R_{CH_4}/R_{C_2H_6}$. On the
basis of the gas-phase mechanism methane arises by

$$CH_3 + CH_3COCH_3 \rightarrow CH_4 + CH_2COCH_3$$

and ethane by

$$2CH_3 \rightarrow C_2H_6.$$
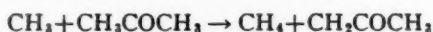
This leads to the constancy of the former ratio. It is evident that this is not
so in the liquid phase. The ratio $R_{CH_4}/R_{C_2H_6}$ remains roughly constant (to
within a factor of about 2) over the intensity range. The ratio $R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}}$,
on the other hand, varies at 40°C. by a factor of about 12, and at all tem-

peratures shows a steady fall with decreasing intensity. However, there are definite indications that the ratio is approaching constancy at high intensities. (See Fig. 1).

(C) Variation of the Rate with Temperature

Table II gives the results of experiments at temperatures from 55°C. to −25°C. at constant intensity. To avoid side reactions the conversion was always kept below 5%.

A plot of log $R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}}$ against $1/T$ (Fig. 2) gives a good straight line and an apparent activation energy of 8 kcal. as compared with 9.7 kcal. for the gas phase. In plotting the results only those at higher intensities were used,

TABLE II

VARIATION OF RATE WITH TEMPERATURE

Temp., °C.	Time, min.	[Ac], molecules/cc. $\times 10^{-21}$	Products, molecules/cc./sec. $\times 10^{-13}$			$\dfrac{R_{CH_4}}{R_{C_2H_6}^{\frac{1}{2}}[Ac]} \times 10^{14}$
			CO	CH$_4$	C$_2$H$_6$	
55	120	7.77	24.3	740	6.9	11.5
55	100	7.77	20.7	565	9.5	7.47
40	120	7.96	16.3	360	5.5	6.81
40.5	109	7.95	14.6	340	4.2	6.61
41	100	7.92	18.2	387	5.1	6.10
33	214	8.05	9.6	229	3.6	4.75
33	210	8.05	13.2	213	3.5	4.46
33	260	8.05	12.0	181	3.2	3.97
15	245	8.26	6.4	84	2.1	2.24
6.5	350	8.37	4.5	38.8	1.44	1.21
6.0	320	8.38	5.2	41.1	1.40	1.31
0.5	200	8.44	4.2	26.2	1.00	0.98
0.5	240	8.44	4.4	26.1	2.00	0.69
−15	1385	8.60	1.8	5.66	0.32	0.37
−16	1215	8.60	1.95	5.46	0.37	0.33
−24	1395	8.65	0.98	2.59	0.21	0.21
−25	1580	8.67	1.57	2.54	0.19	0.21

since the ratio $R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}}$ falls off rapidly with decreasing intensity. Since in most cases the high intensity runs still do not give a quite constant ratio, the results are open to some uncertainty. In view of this the agreement with the gas-phase results is probably satisfactory.

If Arrhenius plots are made of R_{CH_4}, R_{CO}, or $R_{C_2H_6}$ reasonably good straight lines are obtained, from which *apparent activation energies* may be calculated, viz.

$$E_{CH_4} = 11.4 \text{ kcal.}$$
$$E_{CO} = 6 \text{ kcal.}$$
$$E_{C_2H_6} = 7 \text{ kcal.}$$

These have no simple meaning but indicate the order of magnitude of the variations in quantum yield with temperature.

Some experiments with a 1:1 mixture of CH₃COCH₃ and CD₃COCD₃ are shown in Table IIIA. The results agree well with those for light acetone. There is, however, one puzzling feature. In the gas phase it has been found

TABLE IIIA
RUNS WITH A MIXTURE CH₃COCH₃+CD₃COCD₃ IN RATIO 1/1

Temp., °C.	Molecules/cc./sec. × 10⁻¹³								$\dfrac{CH_4+CD_3H}{CD_4+CH_3D}$	
	CD₄	CD₃H	CD₂H₂	CDH₃	CH₄	C₂H₆	CO	CH₄ (total)	Calc.	Exp.
55	34.9	255	42.2	20.2	305	16.0	17.1	657	12.7	10.2
40	18.0	129	2.0	5.9	135	10.2	15.9	290	12.8	11.5
3	2.02	8.4	1.7	2.05	11.8	1.4	2.9	26.0	13.1	4.96
− 3	1.35	4.17	0.78	1.11	6.17	1.32	2.1	13.7	13.12	4.2
*− 3	0.26	1.29	0.23	0.29	1.66	0.26	0.69	3.8		5.4
−24	0.39	1.04	0.18	0.62	1.8	0.19	1.13	4.0	13.24	2.86
*−24	0.068	0.206	0.065	0.065	0.268	0.14	0.57	0.69		3.5

*Runs with 2537 Å.

that there is a higher activation energy for the abstraction of D, as compared with H. On this basis the ratio

$$(CH_4+CD_3H)/(CD_4+CH_3D)$$

should have the values given in the second to last column of Table IIIA. The actual values of the ratio are given in the last column. It will be seen that the ratio falls rapidly at lower temperatures, which is the reverse of the predicted behavior.

The two experiments at 2537 Å give lower rates because of lower light intensity, but show the same trend.

A few experiments were also made with pure CD₃COCD₃, and the results are given in Table IIIB.

TABLE IIIB
RUNS WITH CD₃COCD₃

Temp., °C.	Molecules/cc./sec. × 10⁻¹³		
	CD₄	C₂D₆	CO
55	380	47.4	30.7
20	55.9	6.37	3.3
5	26.5	2.48	0.75
−19	7.35	0.8	0.24

(D) Runs with 2537 Å

The results of a few experiments with λ2537 Å are given in Table IV, and plotted in Fig. 2. The results are rather scattered because of the relatively weaker intensity and therefore smaller amounts of products. In general, however, the trend appears to be very similar. As pointed out above, the quantum yield is not appreciably different at 2537 Å. The results for methane are more accurate than the others because of the larger amounts of products,

and it is possible that the apparent activation energy is a trifle smaller. However, the accuracy of the results is not sufficient to justify any definite conclusion. There thus appears to be no direct evidence for hot radical effects.

TABLE IV
EXPERIMENTS WITH 2537 Å

Time, min.	Temp., °C.	Molecules/cc./sec. ×10^{-12}			$\dfrac{R_{CH_4}}{R_{C_2H_6}^{\frac{1}{2}}[Ac]} \times 10^{14}$
		CO	C$_2$H$_6$	CH$_4$	
140	54	7.9	1.26	131	4.76
330	27	2.8	1.9	74.5	2.10
350	24	4.16	1.55	74	2.30
975	12	1.19	0.34	20.8	1.37
760	10	1.8	0.42	23.0	1.34
840	3	1.15	0.21	13.6	1.12
1580	−23	0.53	0.10	3.05	0.35

(E) Runs with a Solvent

A few experiments were made with water and n-hexane as solvents. The results are given in Tables V and VI. Experiments with D$_2$O showed that the methane formed was entirely CH$_4$.

TABLE V
EXPERIMENTS WITH ACETONE–WATER SOLUTIONS
Temp. = 40°C.

Molecules/cc. ×10^{-21}		Molecules/cc./sec. ×10^{-12}			$\dfrac{R_{CH_4}}{[Ac]} \times 10^{2}$	$\dfrac{R_{CH_4}}{R_{C_2H_6}^{\frac{1}{2}}[Ac]} \times 10^{14}$
[Ac]	[H$_2$O]	CO	CH$_4$	C$_2$H$_6$		
7.92	0	18.2	387	5.1	49.0	6.9
7.17	3.37	4.91	186	4.06	25.8	4.0
5.98	8.44	4.07	194	3.68	32.4	5.3
*3.99	16.8	4.75	181	2.86	45.6	8.5
3.99	16.8	1.29	143	2.30	36.1	7.5

*D$_2$O used in this run.

TABLE VI
EXPERIMENTS WITH ACETONE–n-HEXANE SOLUTIONS
Temp. = 40°C.

Molecules/cc. ×10^{-21}		Molecules/cc./sec. ×10^{-12}			$\dfrac{R_{CH_4}}{[Ac]} \times 10^{2}$	$\dfrac{R_{CH_4}}{R_{C_2H_6}^{\frac{1}{2}}[Ac]} \times 10^{14}$
[Ac]	[Hexane]	CO	CH$_4$	C$_2$H$_6$		
7.92	0	16.3	360	5.5	45.5	6.2
7.92	0	18.2	387	5.1	49.0	6.9
7.85	0.05	5.46	263	4.6	33.5	5.0
7.85	0.05	5.95	256	4.2	32.6	5.1
7.85	0.05	5.74	276	4.5	35.2	5.4
6.4	0.64	2.37	130	2.8	20.2	3.8
5.7	1.29	1.30	65.9	1.6	11.5	2.9
5.7	1.29	1.35	64.0	1.9	11.2	2.6
2.27	3.22	0.76	20.2	0.80	8.9	3.2
1.13	3.86	0.52	8.06	0.74	7.1	2.6

DISCUSSION

There are a number of very puzzling features about the results. It should be emphasized that in view of the high absorption, the distribution of absorbed intensity is very inhomogeneous. As a result the kinetic data can only be assumed to have semiquantitative significance. The discussion will, therefore, be confined to a somewhat qualitative consideration of the main features of the reaction.
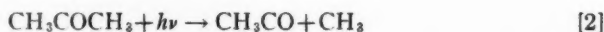
The Quantum Yield

The major difference between the present results and those of the gas-phase photolysis is the low quantum yields. Thus ϕ_{CO} is about 10^{-4} at 25°C. and about 10^{-5} at -25°C. $\phi_{C_2H_6}$ is still smaller. The values of ϕ_{CH_4} are somewhat larger, falling from about 2×10^{-3} at 25°C. to 2×10^{-5} at -25°C. Also, the yield of all reaction products increases very strongly with temperature. Thus over the range -25°C. to 55°C. ϕ_{CH_4} increases by a factor of about 300.

There are two possible explanations of the low quantum yields. In the first place, if the primary step in the gas phase is

$$CH_3COCH_3 + h\nu \rightarrow CH_3COCH_3^* \qquad [1]$$

followed by the decomposition of the excited molecule, it is possible that ϕ will be cut down largely by deactivation in the liquid phase. If this explanation holds deactivation must be strongly temperature dependent. It seems certain that the results are to be explained, at least partially, on the basis of deactivation. There is no question that active molecules play some role in the gas-phase reaction, as indicated by fluorescence studies. There seem to be two types of excited molecule one of which has a life of about 10^{-4} sec. Since there is considerable self quenching which is temperature dependent there seems to be not only collisional deactivation, but also an activation energy for the dissociation of active molecules. The low yields and the effect of temperature on the quantum yields can therefore be attributed at least to some extent to excited molecule deactivation.

The other explanation of the low value of ϕ is recombination because of the cage effect, i.e.

$$CH_3COCH_3 + h\nu \rightarrow CH_3CO + CH_3 \qquad [2]$$

followed by

$$CH_3 + CH_3CO \rightarrow CH_3COCH_3, \qquad [3]$$

the rate of [3] being greatly enhanced by the cage effect. The increase in the quantum yield with temperature is then explained by the increase in the rate of diffusion. A similar explanation has been suggested by Lampe and Noyes (6, 9) to explain the variation of the quantum yield of iodine dissociation in inert solvents. It may be mentioned that reaction [3] may also occur out of the cage. In view of the low quantum yield of ethane formation, however, this must be of minor importance. As mentioned above the apparent activation energy for methane production at 3130 Å is 11.4 kcal. Since methane is by far the largest product, this is approximately the temperature coefficient of the

quantum yield of the over-all decomposition. It is presumably to be related to the temperature coefficient of the diffusion process.

Experiments with a 1:1 mixture of CD_3COCD_3 and CH_3COCH_3 and with pure CD_3COCD_3 give results in general agreement with those with CH_3COCH_3.

The Dissociation of Acetyl by Energy Carry-over

In the gas-phase photolysis it is postulated that, depending on wave length, between 0.07 and 0.22 of the acetyl radicals formed dissociate spontaneously,

$$CH_3CO^* \rightarrow CH_3 + CO. \qquad [4]$$

If this were so in the present case ϕ_{CO} could not be nearly as low as it is. This suggests either that deactivation of an excited molecule is the main cause of the low quantum yield, or else that in the liquid phase most hot acetyls are deactivated and that [4] rarely occurs. This is supported by the fact that ϕ_{CO} has a considerable temperature coefficient.
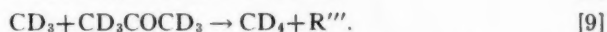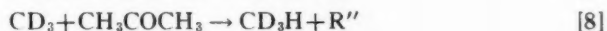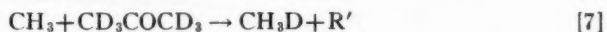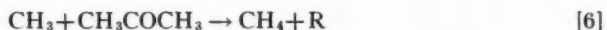
Disproportionation

In the gas phase there is evidence that reaction [5]

$$CH_3 + CH_3CO \rightarrow CH_4 + CH_2{=}CO \qquad [5]$$

occurs about 1/10 to 1/100 as often as [3], and also has an activation energy of about zero. If this were true in the liquid phase it would be impossible to obtain a very low value of ϕ_{CH_4}, unless this were due to deactivation. Also, reaction [5] would lead to E_{CH_4} approaching zero at low temperatures which is far from the case. It seems probable that in the liquid phase where the excited complex $CH_3COCH_3^*$ formed by [3] or [5] will have a very short life, disproportionation may well become negligible compared with recombination. These conclusions are, however, incompatible with those of the following paragraphs concerning deuterium exchange. It is therefore possible that the low quantum yields are due to deactivation of an excited molecule rather than to primary recombination.

It is also possible that disproportionation may occur outside the cage between radicals which have been formed from different molecules. As a check on this a 1:1 mixture of CH_3COCH_3 and CD_3COCD_3 was photolyzed. If the gas-phase mechanism holds almost all methane formed will arise from the abstraction reactions

$$CH_3 + CH_3COCH_3 \rightarrow CH_4 + R \qquad [6]$$

$$CH_3 + CD_3COCD_3 \rightarrow CH_3D + R' \qquad [7]$$

$$CD_3 + CH_3COCH_3 \rightarrow CD_3H + R'' \qquad [8]$$

$$CD_3 + CD_3COCD_3 \rightarrow CD_4 + R'''. \qquad [9]$$

In the gas phase there is little difference between the rates of abstraction by CH_3 and CD_3, but there is considerable difference between the abstraction of an H- or a D-atom. The ratio $(CH_4 + CD_3H)/(CD_4 + CH_3D)$ can be calculated from known rate constants, and will increase slightly at low temperatures as shown by the calculated values in Table IIIA. Actually there is a strong decrease at low temperatures. It should be noted that the ratio is not affected

by uncertainties in the relative ease of splitting off CD_3 or CH_3 from an active molecule. It depends solely on the relative ease of abstracting an H- or a D-atom in the subsequent abstraction reactions.

If methane is also formed by disproportionation between CH_3 or CD_3 radicals and CH_3CO and CD_3CO, since all the disproportionations occur with zero activation energy, the ratio of methanes from disproportionation will be unity. Hence the above ratio will tend toward unity at low temperatures where abstraction is negligible, and will rise rapidly with temperature and approach the 'calculated' values in Table IIIA. The observed behavior thus indicates that disproportionation is becoming more and more important at low temperatures.

The Abstraction Reaction

In the gas-phase photolysis of acetone the methane and ethane formed are almost all accounted for by the reactions:

$$CH_3 + CH_3COCH_3 \rightarrow CH_4 + CH_2COCH_3 \qquad [6]$$

$$CH_3 + CH_3 \rightarrow C_2H_6 \qquad [10]$$
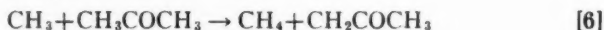
from which

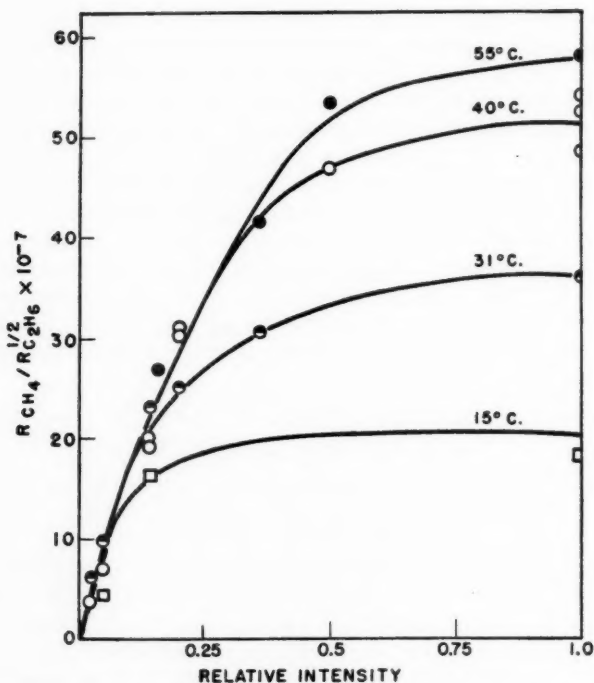$$R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}}[\text{Acetone}] = k_6/k_{10}^{\frac{1}{2}}.$$

FIG. 1.   The variation of $R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}}$ with intensity.

If this relation holds in the liquid phase, $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$ should be independent of the absorbed intensity. In Fig. 1 $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$ has been plotted against the intensity for different temperatures. The figure shows that the ratio decreases sharply with intensity in the low intensity region, although it is tending towards constancy at high intensities. The results suggest that the formation of ethane at low intensity is mainly by some reaction dependent on the first power of the intensity, i.e. of the radical concentration. Possible explanations are formation of ethane to some extent in the cage, or by a direct intramolecular reaction.

The fact that $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$ is nearly independent of intensity at high intensities indicates that under these conditions most methane and ethane is formed by reactions [6] and [10], and that at high intensity inter-cage effects predominate. The photolysis of CD_3COCD_3 furnishes further proof that some ethane is formed by inter-cage effects. In this case the higher strength of C—D bonds reduces the effect of abstraction, and C_2D_6 is greater than CO. This would be impossible if all ethane were formed in the cage.
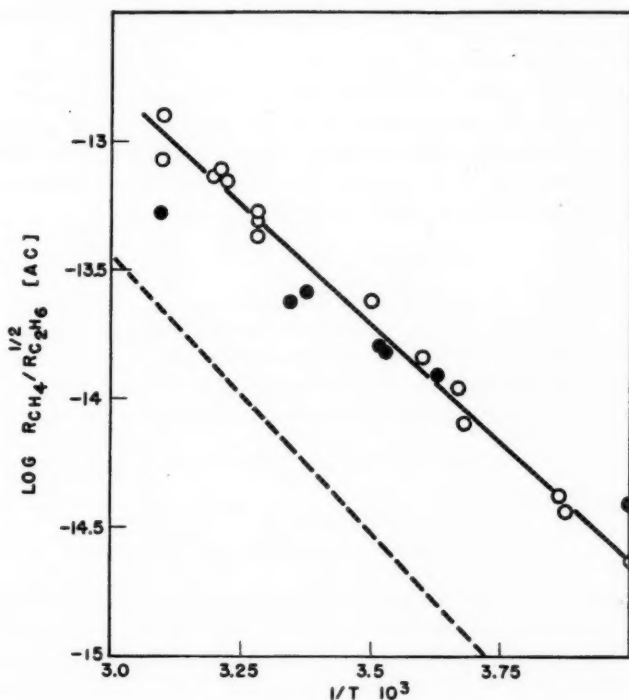

FIG. 2.   Arrhenius plot of $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$ [Acetone] for liquid acetone.
   O   wave length approximately 3130 Å.
   ●   wave length approximately 2537 Å.
The dotted curve represents an extrapolation to low temperatures of results for gaseous acetone.

In Fig. 2 an Arrhenius plot is given of $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$ [Acetone] for runs with CH$_3$COCH$_3$ at 3130 Å and high intensities (i.e. in the region where the ratio is essentially independent of intensity). A good straight line is obtained, from which $E_6 - \frac{1}{2}E_{10} \sim E_6 = 8$ kcal. This agrees satisfactorily with the gas-phase value of 9.7 kcal., since the result will be approximate because of errors due to diffusion effects, to inhomogeneity of absorption, and to the formation of methane by disproportionation of CH$_3$ and CH$_3$CO at low temperatures. While the slopes of the two lines agree reasonably, the gas-phase line lies considerably below that for the liquid phase, corresponding to a difference of a factor of about 7 in the ratio. This has little significance, however, in view of the uncertain meaning of concentration in the liquid phase.

The results obtained by photolyzing CD$_3$COCD$_3$ are no doubt affected to a much greater extent by the occurrence of reaction [5], because of the slower rate of abstraction of D from CD$_3$COCD$_3$ as compared with H from CH$_3$COCH$_3$. The results lead to an apparent activation energy about 2 kcal. lower than those for CH$_3$COCH$_3$, but this is presumably complex and has no simple significance.

The data at 2537 Å are not sufficiently extensive to warrant detailed discussion.

A few experiments were made with acetone–water and acetone–n-hexane solutions, and are given in Tables V and VI. The results indicate:

(a) With water, no change larger than the rather considerable experimental error occurs in the $R_{CH_4}/$[Ac], or $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$ [Ac] ratios. It is evident that water acts essentially as an inert solvent, as far as the production of gaseous products is concerned, and that the reaction

$$CH_3 + H_2O \rightarrow CH_4 + OH$$
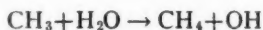
does not occur to an appreciable extent. This is expected in view of the high H—OH bond dissociation energy (ca. 118 kcal.). Previous work (10) indicates that condensable products such as formaldehyde and acetic acid are formed.

(b) With acetone–hexane solutions there is a slight but significant drop in the ratio $R_{CH_4}/R^{\frac{1}{2}}_{C_2H_6}$[Ac]. Since the activation energy of the reaction

$$CH_3 + n\text{-}C_6H_{14} \rightarrow CH_4 + C_6H_{15} \tag{11}$$

is less than that of

$$CH_3 + CH_3COCH_3 \rightarrow CH_4 + CH_2COCH_3 \tag{12}$$

by 1.6 kcal. (11), while the steric factors are in the ratio $P_{11}/P_{12} = 0.3$, it would be expected that abstraction by [11] would be faster than by [12] by a factor of 3.3. A considerable increase in methane would therefore be expected. Actually there is a decrease. However, in view of the probable formation on irradiation of an addition compound between acetone and n-hexane (4) the situation is complex and further discussion is unwarranted.

It is apparent that many features of the results cannot be explained in detail. The above discussion at least points out the general problems involved.

It is hoped that further work on the more complex products, and on the reaction at still lower temperatures, may throw more light on the mechanism.

ACKNOWLEDGMENT

REFERENCES

1. BOWEN, E. J. and HORTON, A. T.   J. Chem. Soc. 1685.   1936.
2. BOWEN, E. J. and PRAUDIERE, E. L. A. E. DE LA.   J. Chem. Soc. 1503.   1934.
3. BOWEN, E. J. and TIETZ, E. L.   J. Chem. Soc. 234.   1930.
4. FRANKENBURG, P. E. and NOYES, W. A., JR.   J. Am. Chem. Soc. 75: 2847.   1953.
5. KASHA, M.   J. Opt. Soc. Amer. 38: 929.   1948.
6. LAMPE, F. W. and NOYES, R. M.   J. Am. Chem. Soc. 76: 2140.   1954.
7. LEIGHTON, P. A., CRARY, R. W., and SCHIPP, L. T.   J. Am. Chem. Soc. 53: 3017. 1932.
8. LEROY, D. J.   Can. J. Research, B, 28: 492.   1950.
9. NOYES, R. M.   J. Chem. Phys. 22: 1349.   1954.
10. QURESHI, U. and TAHER, N. A.   J. Phys. Chem. 36: 2670.   1932.
11. TROTMAN-DICKENSON, A. F. and STEACIE, E. W R.   J. Chem. Phys. 19: 329.   1951.

SUR L'OXYDATION LENTE DE L'ÉTHER DIÉTHYLIQUE EN PHASE GAZEUSE[1]

Par André Lemay[2] et Cyrias Ouellet

RÉSUMÉ

La réaction de l'éther diéthylique avec l'oxygène a été étudiée dans un récipient en Pyrex, dans l'intervalle de température 160-175° C., voisin de la limite inférieure de flamme froide. L'oxygène est consommé durant la baisse initiale de pression suivant une réaction d'ordre zéro, affectée d'une énergie d'activation d'environ 50 kcal./mole, durant laquelle il apparaît de l'eau, des peroxydes, des acides, et autres produits. La hausse de pression qui survient plus tard correspond à des réactions postérieures à l'oxydation proprement dite; elle peut avoir lieu en l'absence d'oxygène. Les taux d'oxydation tirés de la vitesse maximum d'accroissement de la pression sont ainsi remis en question. On discute quelques processus susceptibles d'intervenir dans chacune de ces deux phases de la réaction.

INTRODUCTION

Divers phénomènes caractéristiques de la combustion—oxydation lente, flamme froide, et explosion—peuvent être étudiés dans des conditions particulièrement avantageuses dans l'oxydation de l'éther diéthylique. Ils y sont observables à des pressions basses de l'ordre de 10 à 100 mm. Hg et dans un intervalle de températures allant de 150 à 250° C., tandis que la plupart des hydrocarbures ne subissent ces réactions qu'à des pressions et températures beaucoup plus élevées. Plusieurs aspects de la flamme froide de l'éther ont été étudiés dans notre laboratoire (18) en particulier au moyen du spectromètre de masse à échantillonnage continu de Léger (10, 11). Dans le but de mieux comprendre en quoi consiste le passage de l'oxydation lente à la flamme froide, nous avons cherché dans le présent travail à obtenir de nouvelles données sur la cinétique de l'oxydation lente de l'éther dans l'intervalle de 160 à 175° C. situé immédiatement en dessous de la limite de flamme froide.

L'oxydation des éthers semble occuper une place intermédiaire entre celle des hydrocarbures (5, 12, 15, 21) et celle des aldéhydes (12, 13) mais est moins bien connue que ces dernières. Walsh et ses collaborateurs (4, 14) ont fait une étude détaillée des domaines d'inflammation des éthers, surtout de l'éther diéthylique; mais pour ce qui concerne l'oxydation lente, ces auteurs se sont attachés surtout à celle de l'éther diisopropylique. Eastwood et Hinshelwood (7) ont comparé les oxydations lentes de cinq éthers aliphatiques. Dans le cas de l'éther diéthylique, le plus facilement oxydable de la série étudiée, la réaction débute par une baisse de pression suivie d'un accroissement de pression analogue à celui qui succède à la période d'induction classique des hydrocarbures. En se basant sur cette ressemblance, ces auteurs ont pris comme mesure du taux d'oxydation la pente maximum de cette montée de pression. Ils ont constaté que les peroxydes formés au début de la réaction sont relative-

ment stables et survivent même au passage d'une flamme froide. Il se peut que cette propriété résulte uniquement du fait que la flamme froide de l'éther se produit à une température relativement basse, comparée, par exemple, à celle du butanone (1) durant laquelle les peroxydes accumulés disparaissent rapidement.

MÉTHODE EXPÉRIMENTALE

La réaction est étudiée en système statique en l'absence de vapeur de mercure. La chambre à réaction est un cylindre en Pyrex de 4.4×14 cm. et la pression est enregistrée électriquement.

Appareil

La figure 1 montre un dessin schématique de la chambre à réaction C et de ses principaux accessoires. Elle est enfermée dans un four en acier F chauffé
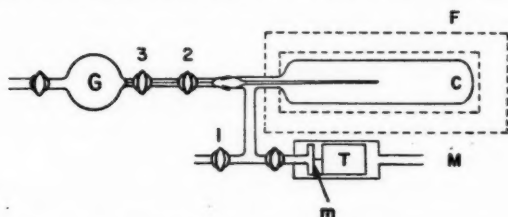

FIG. 1. Schéma montrant la chambre à réaction C, le manomètre à membrane m, et à transducer T, ainsi que le système G-3-2 pour l'injection de gaz étrangers.

électriquement et est entourée d'un manchon en laiton destiné à uniformiser la température. Un thermomètre pénètre dans le four et un couple thermo-électrique peut être déplacé sous le manchon, où le gradient de température ne dépasse pas 1° C. d'une extrémité à l'autre de la chambre à réaction. Un transformateur Sola à voltage constant et un Variac permettent de contrôler la température du four. Le robinet 1 sert à l'introduction des réactifs; il conduit d'une part au réservoir contenant le mélange de gaz et d'autre part à un piège à $-80°$ C. suivi d'une pompe à diffusion utilisant de l'huile silicone et d'une pompe mécanique.

Les tubes qui vont de C aux premiers robinets sont chauffés à 100° C. pour empêcher une condensation qui se produit aux stages ultérieurs de la réaction et qui s'est manifestée dans plusieurs de nos expériences par une baisse considérable de la pression, vers la fin de la réaction.

L'injection, en plein centre de la chambre à réaction, de petites quantités dosées de gaz inhibiteurs, se fait au moyen du tube capillaire, précédé d'un petit volume calibré, inclus entre les robinets 2 et 3, et d'un réservoir G.

La pression est enregistrée au cours de la réaction au moyen d'un manomètre différentiel. Le déplacement d'une membrane m de duralumine de 0.07 mm. d'épaisseur, collée sur une capsule de verre, est transmis à un "transducer" T de type Statham G1-1.5-315, alimenté par une tension de 1.5 volt. Le signal de sortie est transmis à un enregistreur Brown qui trace la courbe de pression. La capsule manométrique et le transducer sont enfermés dans une boîte étanche

en laiton où règne la pression de référence mesurée sur le manomètre anéroide M de type Wallace & Tiernan. Dans la région de 100 mm. Hg qui nous intéresse, la valeur absolue de la pression est mesurée à 0.5 mm. près et les variations sont enregistrées à 0.1 mm. près avec une inertie de l'ordre d'une seconde.

Réactifs

L'éther diéthylique est distillé trois fois en contact avec une solution acide de permanganate, séché en présence de sodium, puis redistillé en rejetant les premières et les dernières fractions. On a aussi employé, avec des résultats identiques, la méthode de Dasler (6) suivant laquelle l'éther est libéré de peroxyde et d'eau par passage à travers une colonne d'alumine activée. Cet éther est conservé sous vide dans un ballon lié à l'appareil. Pour préparer un mélange, on en laisse évaporer une partie dans un ballon évacué, on ajoute la quantité d'oxygène requise, et on laisse reposer pendant quelques heures. Des mesures au spectromètre de masse ont révélé que l'oxygène, provenant directement d'un cylindre, contenait un peu d'azote, mais il a été vérifié que ce gaz n'affecte pas le cours de la réaction. Les robinets sont lubréfiés avec des graisses Apiezon T et L et silicone. Il a été vérifié par des mesures manométriques que ces graisses n'absorbent pas de quantité appréciable d'éther diéthylique, contrairement à ce qui se produit avec les éthers supérieurs.

Parmi les gaz injectés, le formaldéhyde est produit par chauffage de para-formaldéhyde, tandis que l'oxyde nitrique, le propylène, et l'acide bromhydrique sont de marque Mathieson; on les prélève directement des cylindres.

Analyses

On dose l'oxygène en mesurant sa pression partielle dans un analyseur magnétique de Pauling, modèle Beckman D. Au moment choisi, on aspire dans l'analyseur évacué un échantillon (environ 10 cc.) de gaz provenant de la chambre à réaction, après avoir balayé le gaz froid des canalisations en l'aspirant dans un récipient auxiliaire. La quantité de gaz ainsi prélevée est telle qu'il faut interrompre la réaction.

Pour doser les acides et les peroxydes, on évacue la chambre à réaction au moment choisi et on capte dans un piège les produits condensables à −80° C. On extrait ces derniers au moyen de 20 cc. d'eau bidistillée. Les acides sont titrés immédiatement par la soude 0.01 N en présence de phénolphtaléine. Le dosage des peroxydes se fait dans une atmosphère d'azote en titrant par le thiosulfate, 0.01 N d'abord l'iode libéré en 15 min. en présence d'un excès d'iodure de potassium en milieu neutre et, ensuite, l'iode libéré en trois heures en milieu acide.

RÉSULTATS

Marche et produits de la réaction

La réaction est mesurable dans un intervalle de températures restreint; en dessous de 155° C., elle est trop lente, au-dessus de 175° C., elle est trop rapide et tend à dégénérer en flamme froide. La figure 2 montre l'allure générale des phénomènes pour une réaction produite dans des conditions moyennes. Les quantités d'acides et de peroxydes y sont représentées par les pressions

partielles que ces produits exerceraient dans les conditions de l'expérience. Les courbes de pression totale sont reproductibles à 3% près, sauf pour la première expérience d'une série.
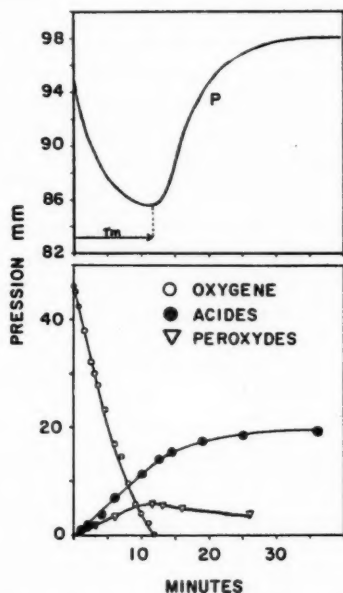

FIG. 2. Courbes de pression totale P et de pressions partielles au cours de la réaction à 168° C. d'un mélange équimoléculaire d'éther et d'oxygène.

Pour le cas typique représenté par la figure 2, la pression totale décroît au début jusqu'à un minimum atteint au bout d'environ 10 min. et se met ensuite à croître pour tendre vers un palier légèrement supérieur à la pression initiale. C'est durant la faible baisse initiale de la pression qu'a lieu la principale réaction d'oxydation, puisque l'oxygène est entièrement consommé durant cette période. Le taux de cette consommation est sensiblement d'ordre zéro. A mesure que l'oxygène disparaît, la pression partielle des produits acides croît linéairement à une vitesse égale à un quart de celle de la consommation de l'oxygène. La concentration des produits peroxydiques croît parallèlement à celle des acides, mais deux fois moins vite. Tout l'iode titrable est libéré rapidement en milieu neutre, ce qui est caractéristique du peroxyde d'hydrogène et des peracides. Après le minimum de pression, les acides augmentent lentement, à un taux sensiblement égal à celui de la diminution des peroxydes.

Des renseignements additionnels sur la variation de la composition du mélange au cours de la réaction ont été fournis par quelques expériences de M. L. P. Blanchard, qui a suivi cette oxydation au moyen d'un spectromètre de masse à échantillonnage continu (10). Les mesures faites avec des mélanges contenant 50 et 30% d'éther indiquent qu'il disparaît deux molécules d'oxygène

pour une d'éther. On observe aussi, dès le début, et jusqu'au moment où le minimum de pression est atteint, un accroissement d'abondance de la masse 18 attribuée à l'eau, et de la masse 43, attribuable à l'acide peracétique ou à l'acide acétique. Rien n'indique une accumulation appréciable de peroxyde d'hydrogène. Durant la montée de pression, on voit croître l'abondance de le masse 28 qui peut refléter, entre autres composés, l'oxyde de carbone. Les résultats de cette étude encore en cours seront publiés plus tard. Des courbes très différentes des précédentes ont été observées dans des expériences sur l'oxydation à 235° C. de l'éther diméthylique par une égale quantité d'oxygène; la réaction débute, sans période d'induction, par un faible accroissement de pression qui se poursuit linéairement jusqu'à un palier.

Influence de la température et de la composition du mélange

La figure 3 fait voir quelques familles de courbes de pression totale et de pression partielle d'oxygène obtenues avec des mélanges de trois compositions différentes, à diverses températures. On voit que le coefficient de température
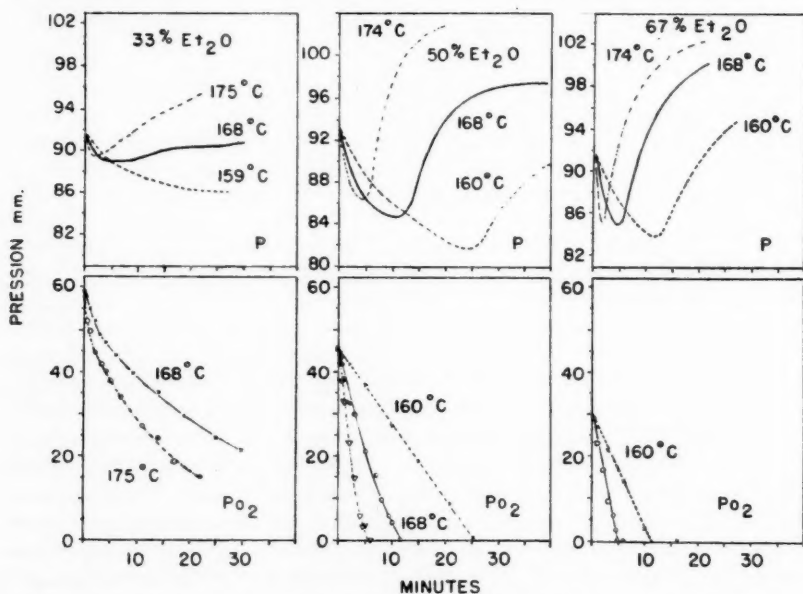

FIG. 3.   Comparaisons de courbes de pression totale (haut) et de pression d'oxygène (bas) pour des mélanges contenant initialement 33%, 50%, et 67% d'éther en volume. (Les courbes de pression totale sont obtenues par enregistrement continu.)

est positif et grand à toutes ces compositions, tant par les taux de descente et de montée de la pression que pour celui de la consommation d'oxygène. Dans les mélanges contenant 50% d'éther ou plus, la consommation d'oxygène semble être d'ordre zéro et est complète au moment où la pression totale passe par le minimum. Les mélanges contenant, au départ, deux fois plus d'oxygène

que d'éther accusent deux particularités: la montée de pression est supprimée aux basses températures; à température plus élevée, la consommation d'oxygène, rapide et apparamment d'ordre zéro jusqu'au minimum, se poursuit ensuite plus lentement et suivant une loi du premier ordre. Cette loi est mise en évidence par la transposition à l'échelle logarithmique des deux courbes obtenues à 168° C. et 175° C.

Comme il n'est pas facile de mesurer sans ambiguité la pente initiale de pression, nous prenons, comme mesure de la vitesse de la première étape de la réaction, soit la pente de la courbe de l'oxygène, soit l'inverse du temps τ_m (figure 2) qui s'écoule entre l'instant initial et celui où le minimum de pression est atteint. La figure 4 montre les droites d'Arrhenius obtenues par ces deux
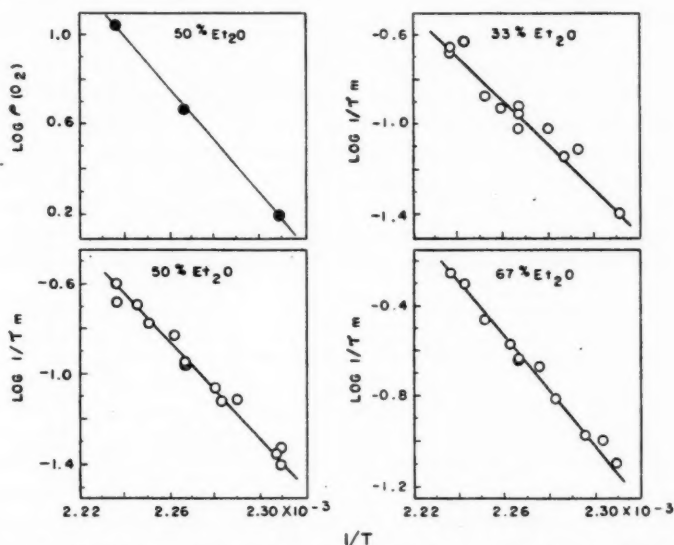

FIG. 4. Courbes de $1/T°$ K. contre les logarithmes de la vitesse initiale calculée d'après le taux de consommation d'oxygène (O_2) et d'après le temps τ_m écoulé au minimum de pression.

méthodes. On a pu vérifier, dans le cas du mélange à 50%, que le coefficient de température obtenu par les deux méthodes est le même, ce qui permet de considérer τ_m comme une grandeur significative. En prenant pour mesure de la vitesse de la seconde étape de la réaction la pente maximum ρ_m de la montée de pression, on obtient les droites d'Arrhenius de la figure 5.

Nos données permettent un calcul approximatif des énergies d'activation apparentes. Pour la phase initiale (figure 4), les valeurs obtenues pour 33%, 50%, 67%, et 90% d'éther sont de 44, 51, 55, et 48 kcal./mole respectivement. La valeur obtenue d'après le taux de consommation d'oxygène à 50% d'éther est 53 kcal./mole, en bon accord avec la valeur ci-dessus tirée de τ_m. Le taux maximum ρ_m d'accroissement de pression conduit à des valeurs de 32, 25, et 26 kcal./mole pour 50, 67, et 90% d'éther respectivement. Ces valeurs gros-

sières, obtenues sur un étroit intervalle de températures, peuvent néanmoins servir à caractériser le coefficient de température près de la limite de flamme froide.
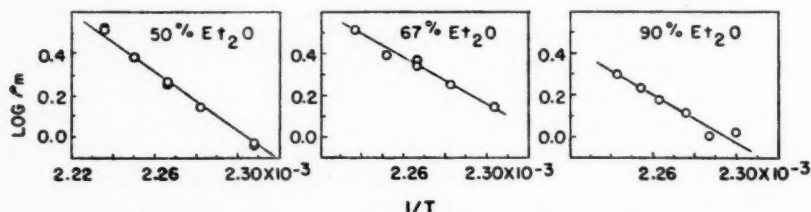

FIG. 5. Courbes de $1/T°$ K. contre le logarithme du taux maximum ρ_m de l'accroissement de pression.

L'influence de la proportion des réactifs sur les deux vitesses représentées par $1/\tau_m$ et ρ_m se voit dans la figure 6. On voit que ces grandeurs sont sensiblement indépendantes de la composition tant qu'il y a moins de 50% d'éther, mais qu'elles varient rapidement dans les mélanges pauvres en oxygène. On a aussi trouvé que, pour un mélange à 50% à 170° C., $1/\tau_m$ varie peu avec la pression totale entre 45 et 95 mm. Hg tandis que ρ_m est sensiblement proportionnel à cette pression. Des expériences effectuées dans le dessein de dégager l'influence de la pression partielle de chacun des réactifs ont abouti à des
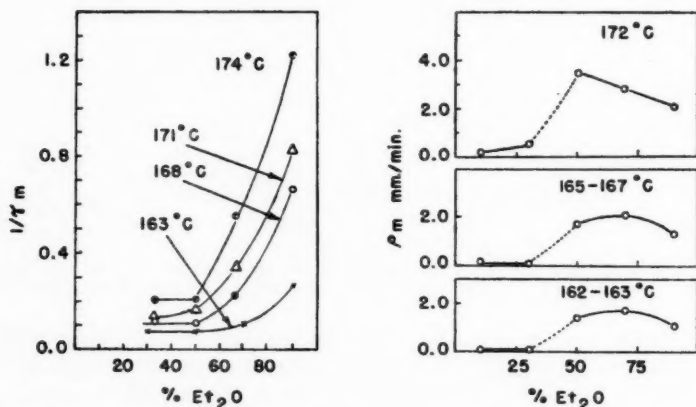

FIG. 6. Variations de la vitesse initiale $1/\tau_m$ min.$^{-1}$ et du taux maximum ρ_m de l'accroissement de pression, en fonction de la composition du mélange.

courbes présentant des maxima semblables à ceux de ρ_m dans la figure 6. Il semble donc que la proportion des réactifs soit le facteur le plus important. Dans l'ensemble, ces résultats concordent avec ceux de Eastwood et Hinshelwood (7) qui ont observé une inhibition considérable par l'oxygène; en effet, la figure 6 montre que la réaction est toujours plus lente aux fortes proportions d'oxygène.

Influence de la surface et de gaz étrangers

Un cylindre en quartz de mêmes dimensions que notre chambre à réaction en Pyrex a donné les mêmes courbes de pression et d'oxygène. Par contre, en traitant la paroi de Pyrex par une solution de chlorure de potassium, nous avons obtenu les courbes de la figure 7. Cet effet a déjà été observé par L. Ouellet et C. Ménard (17), qui nous ont suggéré ces expériences. On voit que, dans ces conditions, l'allure de la réaction ressemble à celle des oxydations d'hydrocarbures et de l'éther diisopropylique (4, 12, 14); une courte période d'induction est décelable sur la courbe de pression et sur celle de la consommation de l'oxygène, mais il n'y a plus de minimum de pression.
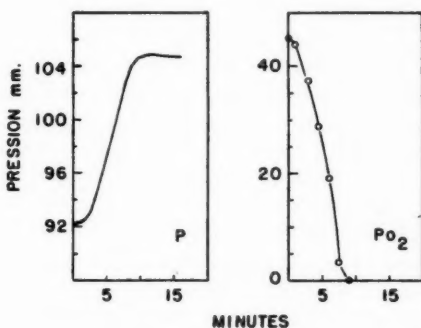

FIG. 7. Courbes de pression totale P et de pression d'oxygène P_{O_2} pour une réaction à 168° C. et 50% d'éther en volume, dans un récipient en Pyrex traité au chlorure de potassium.

L'injection de petites quantités d'oxyde nitrique à diverses époques avant ou durant la réaction inhibe dans tous les cas la consommation de l'oxygène et l'abaissement de la pression, mais n'agit pas toujours de la même façon sur l'accroissement de la pression. La vitesse est réduite d'environ 30 fois par 0.6% d'oxyde nitrique, ce qui donne une indication approximative de la longueur des chaînes. Ces effets et ceux qui résultent de l'addition de propylène, d'acide bromhydrique, et de formaldéhyde seront décrits dans une autre publication.
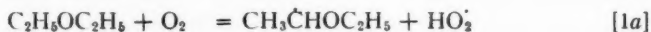
DISCUSSION

La réaction débute sans période d'induction appréciable, contrairement aux oxydations des hydrocarbures et de l'acétaldéhyde; les peroxydes formés n'exercent donc pas d'effet autocatalytique. On distingue deux phases assez nettement séparées dans le temps. La première phase correspond surtout à la consommation de l'oxygène et s'accompagne d'un abaissement de la pression. Sa forte inhibition par l'oxyde nitrique indique qu'il s'agit d'une réaction en chaînes longues. La seconde phase s'accompagne d'un accroissement de la pression; elle n'exige pas la présence d'oxygène libre. Cette seconde phase peut être accélérée, par traitement de la surface en chlorure de potassium, à tel point qu'elle n'est plus en retard sur la précédente; on retrouve alors la courbe de pression ascendante caractéristique des hydrocarbures (figure 7). La compa-

raison des figures 7 et 2 montre que le taux de la consommation de l'oxygène est peu affecté par ce changement de surface.

Cette séparation en deux phases semble constituer la principale différence entre l'oxydation de l'éther diéthylique et celle des hydrocarbures. On peut vraisemblablement l'attribuer à la température anormalement basse d'oxydation de l'éther, plutôt qu'à une différence essentielle de mécanisme; les réactions de la seconde phase seraient suffisamment lentes à cette température pour ne pas masquer la phase initiale d'oxydation. Si cette hypothèse est vraie, on devrait pouvoir reproduire cette séparation en oxydant un hydrocarbure sur une surface empoisonnée, ou la retrouver dans le cas d'hydrocarbures oxydables à température relativement basse. On sait (3) que certaines oléfines semblent se comporter comme l'éther aux basses températures et comme les paraffines aux températures élevées. Dans l'ensemble, l'oxydation lente de l'éther sur le Pyrex et le quartz ressemble beaucoup plus à celle de l'acétaldéhyde, qui débute aussi par une baisse de pression et a lieu dans le même domaine de températures (13), qu'à celle des hydrocarbures (5, 12). Remarquons que la coutume de prendre le taux maximum ρ_m de l'accroissement de pression comme mesure de la vitesse d'oxydation ne peut être suivie dans le cas de l'éther diéthylique; les comparaisons basées sur cette grandeur (7) réflètent plutôt les vitesses des réactions secondaires.
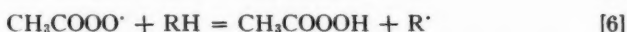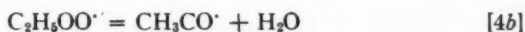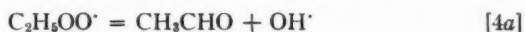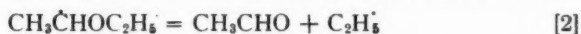
Bien que les faits connus actuellement ne permettent pas une analyse détaillée de la cinétique et du mécanisme de la réaction, il est utile de supposer que les processus en jeu dans chacune des phases sont de mêmes types que ceux qui ont été invoqués pour interpréter l'oxydation des hydrocarbures (2, 5, 12, 21). Appliquant la distinction développée systématiquement par Niclause, Combe, et Letort (15, 16), on peut penser qu'en gros l'abaissement de pression représente la partie de la réaction attribuable à l'oxydation proprement dite, et que la partie de l'oxygène consommé dont cet abaissement ne rend pas compte est engagée dans des dégradations oxydantes s'effectuant sans changement de pression. Enfin, l'accroissement de pression correspondrait à des pyrolyses induites par l'oxygène résiduel ou par la décomposition de peroxydes. L'importance relativement faible des variations de pression laisse supposer que la dégradation oxydante prédomine. L'ordre zéro par rapport à l'oxygène peut être considéré comme une manifestation de l'inhibition par un excès de ce réactif. Ce comportement, bien connu chez les réactions de ce type (2, 5, 7), a été attribué (2) à la prédominance d'un processus de rupture par recombinaison de radicaux peroxydiques.

Pour ce qui concerne la nature des produits intermédiaires au cours de la première phase, il faudra rendre compte de l'apparition hâtive de l'eau et d'un peracide. Il est peu probable que le peroxyde dihydroxyéthylique, produit connu (19) de l'oxydation de l'éther liquide à froid, joue un rôle à la température de notre réaction. Le mode d'initiation le plus vraisemblable est l'abstraction d'un hydrogène secondaire par l'oxygène et par les radicaux, en particulier les radicaux peroxydiques produits au cours de la chaîne.

$$C_2H_5OC_2H_5 + O_2 \quad = CH_3\dot{C}HOC_2H_5 + H\dot{O_2} \qquad [1a]$$

$$C_2H_5OC_2H_5 + HO_2^{\cdot} = CH_3\dot{C}HOC_2H_5 + H_2O_2 \qquad [1b]$$

Ce gros radical peut capter une molécule d'oxygène pour former un radical peroxydique $CH_3CH(O\dot{O})OC_2H_5$ et éventuellement l'hydroperoxyde correspondant; il peut aussi se décomposer et donner lieu aux réactions suivantes:

$$CH_3\dot{C}HOC_2H_5 = CH_3CHO + C_2H_5^{\cdot} \qquad [2]$$

$$C_2H_5^{\cdot} + O_2 = C_2H_5OO^{\cdot} \qquad [3]$$

$$C_2H_5OO^{\cdot} = CH_3CHO + OH^{\cdot} \qquad [4a]$$

$$C_2H_5OO^{\cdot} = CH_3CO^{\cdot} + H_2O \qquad [4b]$$

$$CH_3CO^{\cdot} + O_2 = CH_3COOO^{\cdot} \qquad [5]$$

$$CH_3COOO^{\cdot} + RH = CH_3COOOH + R^{\cdot} \qquad [6]$$

où le rôle de RH sera joué au début par l'éther, mais de plus en plus par l'acétaldéhyde, dont l'hydrogène est plus facile à abstraire. Dans la mesure où [4b] est possible contre la concurrence de [4a] et de [7], on obtiendrait, dans les premiers temps de la réaction, le bilan

$$C_2H_5OC_2H_5 + 2O_2 = CH_3CHO + CH_3COOOH + H_2O \qquad [a]$$

qui correspond au rapport observé des taux de consommation de l'éther et de l'oxygène, mais ne comporte pas d'abaissement de pression. Par ailleurs, [4a] et [4b] seront vraisemblablement concurrencées par la réaction

$$C_2H_5OO^{\cdot} + RH = C_2H_5OOH + R^{\cdot}. \qquad [7]$$

On sait que l'hydroperoxyde d'éthyle se décompose sur la surface avec une demi-vie de l'ordre d'une minute, (9) vers 170° C., avec formation d'acétaldéhyde et d'eau

$$C_2H_5OOH \xrightarrow{\ S\ } CH_3CHO + H_2O \qquad [8]$$
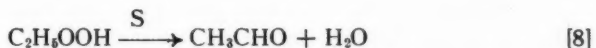
et que cette décomposition est fortement catalysée par le chlorure de potassium. Par la voie [2-3-7-8], on obtiendrait deux molécules d'acétaldéhyde. Dans la région de 160 à 175° C., ce dernier s'oxyde, à une vitesse sensiblement égale (13) à celle de l'oxydation de l'éther, suivant:

$$CH_3CHO + O_2 = CH_3COOOH. \qquad [9]$$

Il est probable que le chlorure de potassium catalyse aussi la décomposition de l'acide peracétique. Ce dernier réagirait aussi dans une certaine mesure avec l'acétaldéhyde, suivant:

$$CH_3COOOH + CH_3CHO = 2CH_3COOH. \qquad [10]$$
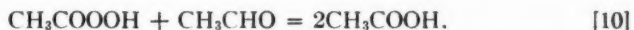
Dans tous les cas envisagés, un fragment de la molécule d'éther donne immédiatement une molécule d'acétaldéhyde suivant (2). L'autre fragment peut être oxydé rapidement en acide peracétique par une seule chaîne [3-4b-5-6]. Ceci donnerait le bilan [a] ne comportant aucune variation de pression. Mais ce second fragment peut aussi donner d'abord une seconde molécule d'acétaldé-

hyde par l'une des voies [4a] ou [3-7-8] puis de l'acide peracétique par [9]. Le faible abaissement de pression réfléterait l'importance relative de la réaction [9]. Enfin, la réaction [10] expliquerait que la quantité d'acide dépasse nettement celle du peroxyde et s'accroît aux dépens de cette dernière.

Au début de la seconde phase, s'il reste beaucoup d'oxygène, la pression continue à décroître à cause de la réaction [9] si la température est assez basse (figure 3). Mais à température plus élevée ou dans un mélange pauvre en oxygène, les réactions de pyrolyse de l'acétaldéhyde (et autres produits) prédominent et la pression croît. Dans ces conditions, la réaction [5] cède le pas à la décomposition du radical acétyle, affectée d'une énergie d'activation de l'ordre de 15 kcal./mole (20):

$$CH_3CO^. = CH_3^. + CO.$$

Le radical méthyle pourra, en l'absence d'oxygène, former du méthane par abstraction et de l'éthane par recombinaison; en présence d'un peu d'oxygène, il sera surtout oxydé suivant l'un des schémas proposés par Grumer (8), avec formation d'oxyde de carbone, de méthanol, et d'eau.

Il est difficile de dire dans quelle mesure la décomposition d'un peroxyde contribue directement à l'accroissement de la pression. Il se peut qu'une telle décomposition gouverne la vitesse de la seconde phase, dont l'énergie d'activation globale est du bon ordre de grandeur, soit de 25 à 30 kcal./mole.

En somme, la première phase correspondrait dans l'ensemble à la dégradation en molécules à deux atomes de carbone, la seconde phase, à celle en molécules à un atome de carbone. En présence de chlorure de potassium (figure 7), la décomposition de l'acide peracétique et éventuellement de l'hydroperoxyde d'éthyle serait accélérée à tel point que la seconde phase ne serait plus en retard sur la première.

La comparaison du taux d'oxydation lente de l'éther à ceux d'autres substances est impossible du fait que la pente maximum ρ_m de la courbe d'accroissement de pression a été prise par plusieurs auteurs comme mesure des taux d'oxydation lente de diverses substances organiques. Or, dans le cas de l'éther et vraisemblablement dans plusieurs autres, cette grandeur n'a pas la signification qu'on lui avait prêtée.

SUMMARY

The reaction of diethyl ether and oxygen in a Pyrex vessel has been studied over the temperature range 160–175° C., below the lower limit of cool flame. During the initial pressure drop, oxygen is consumed in a zero order process with an activation energy of some 50 kcal./mole, giving rise to water, peroxides,

acids, and other products. The pressure rise which follows is secondary to the main oxidation reaction; it does not require the presence of oxygen. Oxidation rates derived from maximum rates of pressure rise are therefore questionable. A number of processes, which may account for these two reactions stages, are examined.

RÉFÉRENCES

1. BARDWELL, J. et HINSHELWOOD C.   Proc. Roy. Soc. (London), A, 205: 375.   1951.
2. BATEMAN, L.   Quart. Revs. (London), 8: 147.   1954.
3. BAWN, C. E. H. et SKIRROW, G.   Abstr. Fifth Symposium on Combustion.   Pittsburg. 1954.   p. 82.
4. CHAMBERLAIN, G. H. N. et WALSH, A. D.   Flame and Explosion Phenomena.   Third Symposium on Combustion.   The Williams & Wilkins Company, Baltimore.   1949. p. 368.
5. CULLIS, G. H. N. et HINSHELWOOD, C. N.   Discussions Faraday Soc. No. 2: 117.   1947.
6. DASLER, W. et BAUER, C. D.   Ind. Eng. Chem. Anal. Ed. 18: 52.   1946.
7. EASTWOOD, T. A. et HINSHELWOOD, C.   J. Chem. Soc. 733.   1952.
8. GRUMER, J.   Abstr. Fifth Symposium on Combustion.   Pittsburg.   1954.   p. 41.
9. HARRIS, E. J.   Proc. Roy. Soc. (London), A, 173: 126.   1939.
10. LÉGER, E. G.   Can. J. Phys. 33: 74.   1955.
11. LÉGER, E. G. et OUELLET, C.   J. Chem. Phys. 21: 1310.   1953.
12. LEWIS, B. et VON ELBE, G.   Combustion, flames and explosion of gases.   Academic Press, Inc., New York.   1951.
13. MACDOWELL, C. A. et THOMAS, J. H.   J. Chem. Soc. 2217.   1949.
14. MALHERBE, F. E. et WALSH, A. D.   Trans. Faraday Soc. 46: 824.   1950.
15. NICLAUSE, M. et COMBE, A.   Rev. inst. franç. pétrole, 8: 311.   1953.
16. NICLAUSE, M., COMBE, A., et LETORT, M.   J. chim. phys. 49: 605.   1952.
17. OUELLET, L. et MÉNARD, C.   Communication privée.
18. OUELLET, L. et OUELLET, C.   Can. J. Chem. 29: 986.   1951.
19. RIECHE, A.   Z. angew. Chem. 44: 896.   1931.
20. STEACIE, E. W. R.   Atomic and free radical reactions.   2nd ed.   Reinhold Publishing Corporation, New York.   1954.
21. WALSH, A. D.   Trans. Faraday Soc. 43: 305.   1947.

POLYMERIZATION OF 2-FLUOROBUTADIENE-1,3 AND PROPERTIES OF POLYMERS[1]

By R. J. Orr and H. Leverne Williams

ABSTRACT

Fluorobutadiene has been copolymerized with styrene in emulsion at 5°C. The rate of mercaptan consumption, rate of conversion, copolymer composition, and the intrinsic viscosity of the copolymer have been measured. Reactivity ratios and regulating indices were calculated for this temperature. Copolymerization reactions at 50°C. were studied in solution, and the reactivity ratios calculated. These were, for the following monomers as monomer 2: styrene at 5°C., $r_1 = 1.61 \pm 0.24$ and $r_2 = 0.16 \pm 0.08$; styrene at 50°C., $r_1 = 1.55 \pm 0.10$ and $r_2 = 0.50 \pm 0.10$; acrylonitrile at 50°C., $r_1 = 0.59 \pm 0.10$ and $r_2 = 0.07 \pm 0.03$; isoprene at 50°C., $r_1 = 2.05 \pm 0.19$ and $r_2 = 0.19 \pm 0.10$; α-methyl styrene at 50°C., $r_1 = 1.77 \pm 0.19$ and $r_2 = 0.38 \pm 0.11$; and methyl methacrylate at 50°C., $r_1 = 1.54 \pm 0.08$ and $r_2 = 0.64 \pm 0.08$. Polyfluorobutadiene has a cohesive energy density of 90-100 cal./cc., a second order transition point of -62.5°C., and some units formed by 1.2 or 3.4 addition.

INTRODUCTION

There are few data on the polymerization of 2-fluorobutadiene or on the physical properties of its polymers. The patent literature contains information on preparation of the polymers in emulsion and their properties (8, 9, 10, 16, 17, 18, 19, 24). Other papers have appeared summarizing the properties and preparations of the polymers (21, **33**).

Fluorobutadiene lies between butadiene and chlorobutadiene in its properties. It polymerizes nearly as rapidly as chlorobutadiene, but unlike the latter copolymerizes with many monomers readily. Homopolymers have been found to be resistant to crystallization on stretching but require reinforcement for development of high tensile properties. Methods of synthesis of the monomer have been reported in a number of patents (2, 11, 12, 25, 26, 27, 28, 29).

EXPERIMENTAL RESULTS

Copolymerization Rates

The rate of conversion was measured as a function of monomer ratio for copolymerization with styrene at 5°C.; the results are given in Table I. The conversion rate is unaffected by the per cent diene in the charge. The time–conversion relation is quite linear, showing a rate of conversion considerably more rapid than during the polymerization of butadiene.

Chain Transfer Reactions

The intrinsic viscosities of the polymers prepared to different conversions, from various charge ratios and mercaptan contents, were measured in benzene and ethylene dichloride. The mercaptan used was a mixture of tertiary mercaptans of an average molecular weight 220. The data are in Table II. The disappearance of mercaptan (RSH) over the course of the polymerization was determined by amperometric titration. The results are in Table III.

TABLE I

CONVERSION RATES FOR STYRENE–FLUOROBUTADIENE COPOLYMERIZATION

% Diene in charge	Hours	% Conversion
100	3.75	43
78	1.00	11
	2.50	40
60	1.00	18
	1.50	26
	2.25	36
	3.25	49
20	1.00	10
	3.00	40

TABLE II

INTRINISIC VISCOSITIES OF FLUOROBUTADIENE POLYMERS AND COPOLYMERS

% Diene in charge	Mercaptan parts	% Conv.	Solvent	$[\eta]$
100	0	18		0.49
		23		0.50
		30		0.49
		36	Ethylene dichloride	0.67
		38		0.50
		45		0.50
		48		0.84
		58		0.43
		43		0.3
	0.9	46		0.6
	2.0	40		0.7
78	2.3	18	Benzene	0.90
		34		0.88
		48		0.65
60	2.3	21		0.80
		38		0.79
		49		0.80
20	2.3	14		0.4
		36		1.0
		42		0.7

TABLE III

MERCAPTAN DISAPPEARANCE IN STYRENE–FLUOROBUTADIENE
COPOLYMERIZATION

% Diene	% Conversion	[RSH]/[RSH]$_0$
78	18	0.79
	34	0.31
	48	0.36
60	21	0.85
	38	0.61
	49	0.33

Polymer Compositions from Emulsion and Solution Polymerizations

Polymers from polymerizations in emulsion and solution, at 5° and 50°C. respectively, were analyzed for per cent diene.

Styrene was the comonomer at 5°C. and the comonomers at 50°C. were styrene, acrylonitrile, isoprene, α-methyl styrene, and methyl methacrylate.

The emulsion polymers were obtained at varying conversions. The polymers prepared in solution were as low conversion as possible. The results of the analyses are in Table IV.

TABLE IV

POLYMER COMPOSITIONS FOR FLUOROBUTADIENE (M_1) COPOLYMERS

Weight % M_1 in initial monomer		% Conv.	Weight % diene	Weight % diene at 0 % conv. (calc.)
78	M_2 = styrene at 5°C.	33	87.5	87.2
		47	87.0	
60		11	72.0	72.5
		15	71.5	
		38	73.0	
		49	73.0	
30		6.5	53.5	53.5
		30	53.5	
		50	51.2	
20		14	41.0	41.0
		36	33.0	
		42	31.0	
60	M_2 = styrene at 50°C.	<3	73.9	75.3
		<3	74.6	
		<3	78.6	
50		<3	62.2	61.3
		<3	60.5	
40		<3	55.8	52.7
		<3	48.1	
20		<4	38.0	37.4
		<4	36.8	
68	M_2 = α-methyl styrene at 50°C.	6.36	81.9	80.0
		13.78	78.0	
52		2.66	67.9	68.5
		11.37	69.0	
36		2.20	47.9	50.8
		8.55	43.8	
20		1.19	31.2	36.0
		5.28	40.6	
68	M_2 = methyl methacrylate at 50°C.	8.12	76.2	78.1
		11.60	80.0	
52		10.17	50.3	56.9
		11.23	63.5	
36		9.97	48.2	49.5
		15.27	50.8	
20		15.60	20.5	27.3
		17.36	34.2	
68	M_2 = isoprene at 50°C.	<4	85.7	83.8
		12.7	81.9	
52		6.8	73.7	73.7
36		5.3	65.2	65.2
20		<4	38.4	40.8
		<4	43.2	
60	M_2 = acrylo- nitrile at 50°C.	4.7	67.6	67.6
50		2.3	63.6	63.6
40		5.0	61.1	61.1
20		6.9	50.4	50.4

Effect of Solvents on Vulcanized Polyfluorobutadiene

The tendency of this polymer to swell in a series of solvents of varying cohesive energy densities was determined. The per cent swell was determined by soaking a strip of the rubber in the solvent and determining the weight increase. The equilibrium weight was used to determine the amount of swelling.

Some of the cohesive energy density values were taken from publications (14, 31). Those unavailable were calculated from the relation c.e.d. $= \Delta H_{vap} - RT$. In Table V are given the results.

TABLE V

SWELL BEHAVIOR OF VULCANIZED POLYFLUOROBUTADIENE

Solvent	c.e.d. (Ref.)	% Swell
Toluene	80 (14)	115
Benzene	82 (14)	110
Methyl ethyl ketone	86	93
Ethylene dichloride	96	219
Carbon disulphide	97	134
Acetone	100 (14)	110
Pyridine	100 (31)	203
Dioxane	100 (14)	199
Dimethyl formamide	140 (31)	100
Nitromethane	160 (31)	183

Second Order Transition Temperature

This was determined by a dilatometric method as being −62.5°C. There was no evidence of crystallization at this temperature.

Infrared Spectra of Polyfluorobutadiene

All infrared spectroscopic work had to be done on the solid films since the polymers were insoluble in carbon disulphide. No quantitative conclusions were possible but qualitative results could be obtained. In Fig. 1 are curves for polyfluoro- and polychloro-butadiene. These are compared with the spectra of polybromoprene (20). Since all spectra were determined in polyethylene, no data were obtained below 7.8 microns where polyethylene begins to interfere.
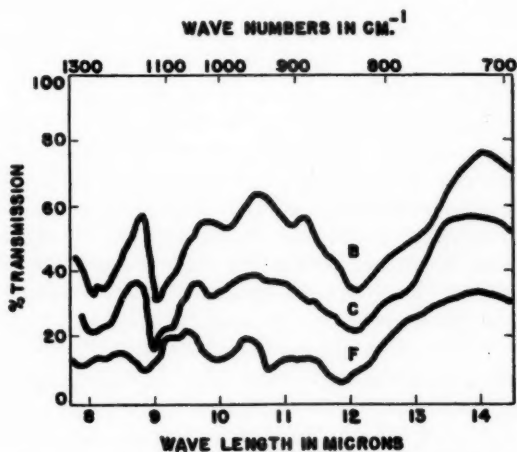
FIG. 1.   Infrared spectra of polyfluorobutadiene (F), polychlorobutadiene (C), and polybromobutadiene (B).

DISCUSSION

Emulsion Polymerizations

Emulsion copolymerization rates are quite rapid compared to butadiene (3, 6) indicating either efficient utilization of initiating radicals or a high solubilization of monomers by soap. The independence of rate with per cent fluorobutadiene in the charge suggests polymerization rates similar to those of styrene. The low intrinsic viscosities of the polymers may be due to low molecular weight, a highly gelled polymer, or an improper choice of solvent. Since the values did not change markedly for measurements using two liquids of a different cohesive energy density, the choice of solvent could not have been responsible. There seems little possibility that the polymers were gelled since the intrinsic viscosity of polyfluorobutadiene prepared in the absence of mercaptan did not show the peak characteristic of gelation, even at very low conversion. The low intrinsic viscosities must be characteristic of low molecular weights due to chain transfer reactions. These cannot be due to mercaptan since the intrinsic viscosity is independent of the mercaptan content of the original charge. Either the fluorobutadiene itself, or some impurity difficult to remove, such as difluorobutene, must be responsible. The mercaptan does react during polymerization at a rate indicating the same reactivity toward the fluorobutadienyl radical as toward butadienyl (6). If this reaction is first order with respect to mercaptan over the first part of the reaction, the regulating indices would be 1.8 and 0.6 for 78/22 and 60/40 fluorobutadiene–styrene charge ratios respectively.

Structure of Polyfluorobutadiene

The dominant band in the spectra near 12 microns must be due to the C–H on the CH=CF group. This completely obscures that portion of the spectra which would give a measure of the proportion of *cis* 1,4 addition (by analogy with the spectrum of Hevea). Some 1,2 or 3,4 addition is indicated by the band at 10.75 microns. This is different from polychlorobutadiene which is 100% 1,4 (20).

Solubility of Polyfluorobutadiene

One method of determining the cohesive energy density (c.e.d.) of a polymer is to find the liquid in which the swelling of the vulcanized polymer is a maximum (31). From the data in Table V, the cohesive energy density of polyfluorobutadiene was estimated as 90–100 cal./cc. Acetone and nitromethane gave anomalous results. The cohesive energy density is of the order of that for polyvinyl chloride and is larger than the value for polychlorobutadiene and 75/25 butadiene–acrylonitrile copolymers. Fluorine changed the cohesive energy density of the polymer in the same direction but to a greater extent than did chlorine. This does not correlate well with the low values obtained with other fluorine containing polymers (31). The structure of the polymer chain may affect the part that fluorine plays in determining the cohesive energy density.

Reactivity Ratios of Fluorobutadiene in Copolymerization

The reacted monomer data from the emulsion polymerizations were extra-

polated to zero conversion. Solution polymerizations were stopped at low conversions and the polymer composition assumed to be identical with that formed at zero conversion. These values, with the corresponding charge ratio, were substituted in the copolymerization equation, the r_1–r_2 lines calculated, and the values of r_1 and r_2 determined. The error was estimated from the size of the triangle of intersection. A typical r_1–r_2 plot is shown in Fig. 2. In Table VI are the values which were determined.
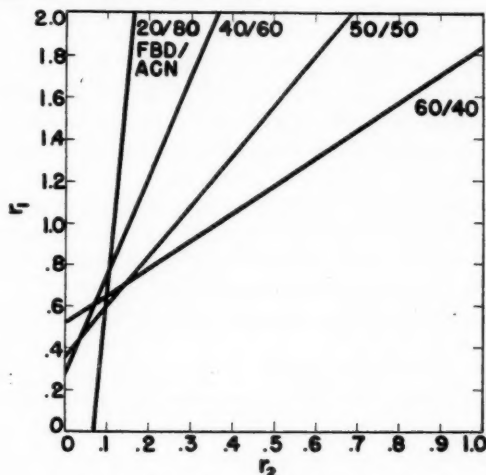
FIG. 2. r_1–r_2 lines for fluorobutadiene–acrylonitrile copolymerization at 50°C.

TABLE VI

REACTIVITY RATIOS OF FLUOROBUTADIENE WITH COMONOMERS

Comonomer (M_2)	T (°C.)	r_1	r_2	$r_1 \times r_2$
Styrene	5	1.61±0.24	0.16±0.08	0.26
Styrene	50	1.55±0.10	0.50±0.10	0.78
Acrylonitrile	50	0.59±0.10	0.07±0.03	0.04
Isoprene	50	2.05±0.19	0.19±0.10	0.39
Alpha methyl styrene	50	1.77±0.19	0.38±0.11	0.67
Methyl methacrylate	50	1.54±0.8	0.64±0.08	0.99

The influence of temperature on r_2 seems much more pronounced than on r_1. There is a strong tendency to alternate in the copolymers, reaching a maximum with acrylonitrile and a minimum with methyl methacrylate. The ratio of r_1 and r_2 indicates a general tendency to enter the copolymer more rapidly than the comonomer.

The Q and e Values of Fluorobutadiene

These values were calculated from the Alfrey-Price equation and are in Table VII. The values for fluorobutadiene were determined using styrene as the primary standard with $Q = 1.0$ and $e = -0.8$. Those for the other comonomers were determined using the fluorobutadiene as a secondary standard.

TABLE VII

Q AND e VALUES FOR FLUOROBUTADIENE AND THE COMONOMERS

Monomer	T (°C.)	Q	e
Fluorobutadiene	5	1.39	+0.36
Fluorobutadiene	50	1.32	−0.30
Methyl methacrylate	50	0.85	−0.30
Acrylonitrile	50	2.5	+1.5
Isoprene	50	0.86	−1.27
Alpha methyl styrene	50	0.98	−0.93

Agreement with the values reported previously (1) is within experimental error except for the e value of methyl methacrylate which was reported as 0.4. The Q and e values for isoprene at 50°C. were not previously reported but the deviation from butadiene at this temperature is in accord with that observed at −18°C. (22, 23).

It is of interest to compare the relative effects of chlorine and fluorine substitution on the polarity of the double bond and the resonance stabilization of the dienyl free radical. The reactivity ratios for chlorobutadiene have been reported as $r_1 = 3.41$, $r_2 = 0.59$ for butadiene and $r_1 = 3.65$, $r_2 = 0.133$ for isoprene (chlorobutadiene being monomer-1) at 50°C. (30). Using butadiene as a secondary standard with $Q = 1.33$ and $e = −0.8$, then Q and e for chlorobutadiene are 1.5 and −0.24 respectively. It may be seen that the change in Q and e caused by the substitution of the fluorine group is in the same direction, but not as pronounced as it was for the chlorine. Fluorobutadienyl radical is less resonance stabilized and the double bond of the monomer is poorer in electrons than that of the chlorobutadiene.

EXPERIMENTAL METHODS

The recipe was similar to one developed previously for butadiene copolymerization (6) and is:

Monomers	100 parts by weight, active material
Water	180
Potassium fatty acid soap flakes	5.0
Mixed tertiary mercaptans	Variable
Cumene hydroperoxide	0.10
Ferrous sulphate heptahydrate	0.14
Potassium pyrophosphate	0.177

An emulsion of ditertiary butyl hydroquinone was used as the stopping agent.

Fluorobutadiene was prepared by the Defence Research Chemical Laboratory through the courtesy of Dr. H. Sheffer. It was subjected to simple distillation before use. Other monomers were commercial materials which were fractionated, taking the center fraction. All polymerization chemicals were standard Commercial grade. Analytical reagents were C.P. grade.

The mercaptan in the latex was determined by amperometric titration (13). Intrinsic viscosities were measured at 30.0°C. by dissolving the latex directly in an 80:20 benzene–ethanol or ethylene dichloride – ethanol solution. Dilutions were made with pure benzene (or ethylene dichloride) so that intrinsic viscosities could be calculated (4, 7, 15).

The polyfluorobutadiene was synthesized at 5°C., coagulated in brine acid, stabilized with BLE, compounded in the test recipe, and vulcanized. Swelling indices were done on the vulcanized stock. Second order transition points were determined on uncured samples by a dilatometric technique. Polymers were purified for analysis by swelling in benzene and extracting in methanol.

A normal Parr bomb combustion using 50 mgm. polymer, 15 gm. sodium peroxide, and 0.5 gm. benzoic acid was used. The ignition was electrical and it was found that the position of the fuse wire in respect to the top of the combustion mixture was critical. Potassium perchlorate was omitted from the combustion mixture since it was found to have a deleterious effect in the subsequent colorimetric analysis for fluoride ion.

Fluoride was determined on aliquots of the solution containing the soluble combustion products by the extent of bleaching brought about by the addition of fluoride ions to a ferric thiocyanate solution (5, 32). The amount of bleaching is definite and reproducible but not a linear function of fluoride ion concentration. There is a slow but distinct decrease in the colorimeter reading with time and the color varies considerably with pH. The combustion mixture was brought to pH = 7.0 with nitric acid and further adjusted to 2.0 with hydrochloric acid. The method was standardized against sodium fluoride solution with readings taken on a Klett-Summerson colorimeter and a Beckman DU ultraviolet spectrophotometer set at 4900 Å wavelength. Analysis of polyfluorobutadiene indicated complete recovery and identification of organic fluorine.

ACKNOWLEDGMENT

REFERENCES

1. ALFREY, T. and PRICE, C. C. J. Polymer Sci. 2: 101. 1947.
2. COFFMAN, D. D. and SALISBURY, L. F. (duPont) U.S. Patent No. 2,451,612. Oct. 19, 1948.
3. EMBREE, W. H., SPOLSKY, R., and WILLIAMS, H. L. Ind. Eng. Chem. 43: 2553. 1951.
4. FORDHAM, J. W. L., O'NEIL, A. N., and WILLIAMS, H. L. Can. J. Research, F, 27: 119. 1949.
5. FOSTER, M. D. Ind. Eng. Chem. Anal. Ed. 5: 234. 1933.
6. GILBERT, R. D. and WILLIAMS, H. L. J. Am. Chem. Soc. 74: 4114. 1952.
7. HENDERSON, D. A. and LEGGE, N. R. Can. J. Research, B, 27: 666. 1949.
8. HILL, F. B., Jr. (duPont) U.S. Patent No. 2,436,213. Feb. 17, 1948.
9. HILL, F. B., Jr. (duPont) Can. Patent No. 459,661. Sept. 13, 1949.
10. IMPERIAL CHEM. INDUSTRIES Brit. Patent No. 591,086. Aug. 6, 1947.
11. IMPERIAL CHEM. INDUSTRIES Brit. Patent No. 603,855. June 24, 1948.
12. JOHNSON, F. W. (duPont) U.S. Patent No. 2,398,181. April 9, 1946.
13. KOLTHOFF, I. M. and HARRIS, W. E. Ind. Eng. Chem. Anal. Ed. 18: 161. 1949.
14. MARK, H. and TOBOLSKY, A. V. Physical chemistry of high polymeric systems. Vol. 2. Interscience Publishers, Inc., New York. 1940. p.261.
15. MITCHELL, J. M. and WILLIAMS, H. L. Can. J. Research, F, 27: 35. 1949.
16. MOCHEL, W. E. (duPont) U.S. Patent No. 2,426,560. Aug. 26, 1947.
17. MOCHEL, W. E. (duPont) U.S. Patent No. 2,429,838. Oct. 28, 1947.
18. MOCHEL, W. E. (duPont) U.S. Patent No. 2,446,382. Aug. 3, 1948.
19. MOCHEL, W. E. (duPont) Can. Patent No. 459,955. Sept. 27, 1949.

20. MOCHEL, W. E. and HALL, M. B.   J. Am. Chem. Soc. 71: 4082.   1949.
21. MOCHEL, W. E., SALISBURY, L. F., BARNET, A. L., COFFMAN, D. D., and MIGHTON, C. J. Ind. Eng. Chem. 40: 2285.   1948.
22. ORR, R. J. and WILLIAMS, H. L.   Can. J. Chem. 29: 270.   1951.
23. ORR, R. J. and WILLIAMS, H. L.   Can. J. Chem. 30: 108.   1952.
24. SALISBURY, L. F.   (duPont) U.S. Patent No. 2,416,456.   Feb. 25, 1947.
25. SALISBURY, L. F.   (duPont) U.S. Patent No. 2,426,792.   Sept. 2, 1947.
26. SALISBURY, L. F.   (duPont) U.S. Patent No. 2,437,307.   Mar. 9, 1948.
27. SALISBURY, L. F.   (duPont) U.S. Patent No. 2,437,308.   Mar. 9, 1948.
28. SALISBURY, L. F.   (duPont) U.S. Patent No. 2,469,948.   May 10, 1949.
29. SALISBURY, L. F.   (duPont) U.S. Patent No. 2,519,199.   Aug. 14, 1950.
30. SIMHA, R. and WALL, L. A.   J. Research Natl. Bur. Standards, 41: 521.   1948.
31. SMALL, P. A.   J. Appl. Chem. (London), 3: 71.   1953.
32. SMITH, H. V.   Ind. Eng. Chem. Anal. Ed. 7: 23.   1935.
33. STARKWEATHER, H. W., BARE, P. O., CARTER, A. S., HILL, F. B., Jr., HURKA, V. R., MIGHTON, C. J., SANDERS, R. A., WALKER, H. W., and YOUKER, M. A.   Ind. Eng. Chem. 39: 210.   1947.

LCAO MO CALCULATIONS ON SATURATED HYDROCARBONS AND THEIR SUBSTITUTED DERIVATIVES[1]

BY C. SANDORFY[2]

ABSTRACT

The simple semiempirical molecular orbital method was applied to saturated hydrocarbons in three different approximations. Electronic charge distribution diagrams were obtained. Characteristic differences between saturated and conjugated compounds concerning bond localization and the effect of an electronegative substituent can be interpreted on the basis of these diagrams. The $1s$ electrons of the hydrogens are introduced for the first time in semiempirical molecular orbital calculations.

I. INTRODUCTION

The molecular orbital method in its simple form, where the electrons are assigned to one-electron wave functions (ϕ_j) expressed as linear combinations of atomic orbitals

[I]
$$\phi_j = c_1 \psi_1 + c_2 \psi_2 + c_3 \psi_3 + \ldots,$$

was applied with success to the evaluation of energy levels and electronic charge distribution in conjugated molecules (19, 26).

The majority of these calculations were carried out by use of the very flexible Hückel approximation (18) where the π-electrons are supposed to live a somewhat independent life in the averaged field of the nuclei and the core and σ-electrons. Objections against this approximate method are easy to find. The separation of π- and σ-electrons needs justification and recently Altmann and Coulson (1, 8) questioned it. However work by Ross (37) and Moser (25) seems to prove that this approximation is a fair one. Other objections arise owing to the unsufficient account taken of spin and the mutual repulsion of the electrons. Partial improvement in these respects might be attained by more elaborate methods, such as the methods of antisymmetrized molecular orbitals (15), configurational interaction (6, 9), and self-consistent LCAO (36). Nevertheless, in spite of a certain success, these improved methods fail to give a decisively better understanding of the molecules than that obtained by the simpler semiempirical LCAO method. The reason for this lies probably in the fact that these methods require an actual knowledge of the wave functions whereas the semiempirical character of the simpler method makes this unnecessary.

Since 1949 the evolution of theoretical chemistry has taken new directions following the critical work of Lennard-Jones (Ref. 20 and subsequent papers), the new ideas of "the most probable configuration" (2, 22, 39, 43) introduced before by Artmann, and Daudel's new approach to the problem of localizability of electrons (la théorie des loges) (11).

The fact remains, however, that the simple semiempirical LCAO MO method succeeded in giving a fair interpretation of the electronic spectra of

conjugated molecules (5, 27, 33) and of the electronic charge distribution in them (7, 12, 34, 42). The reasons for this surprising success were examined by Mulliken (28) (Roger (35) even tried to deduce this method independently of wave mechanics).

In view of this fact we are compelled to believe that, although apparently very approximate, the semiempirical LCAO MO method constitutes a very useful tool in investigation of molecular structure (see Refs. 13 and 34), and it may be considered as the next stage of evolution after Pauling's resonance theory (14, 30).

II. CALCULATIONS ON SATURATED COMPOUNDS

The LCAO molecular orbital method was first applied to the H_2 molecule, and the H_2^+ ion (see Ref. 31), and there is no reason why its use should be restricted to "mobile" or π-electrons.

There exists however a very serious limitation due to the greater complexity of the problems involved. There is a certain justification in treating π-electrons separately from the others, but, if we turn our attention to the saturated compounds, there is now no reason to neglect any of the orbitals participating in bonds, or the lone pair electrons. All orbitals have to be taken into consideration, except possibly the K orbitals.

Thus, for example, in the case of n-butane we have 26 orbitals instead of 4 in the π-electron treatment of 1,3-butadiene.

This fact is not however prohibitive in every case, because, firstly, symmetry considerations enable us to simplify the secular equations, and, secondly, calculating machines can be used to solve systems of equations of a relatively high degree.

The main points to notice are that, in using a wave function of the form of [I], we do not "delocalize" the electrons, and also that when the molecular orbital is a linear combination of atomic orbitals it bears no relation to the "mobility" of the electrons in certain orbitals.

Lennard-Jones (20) has insisted on the fact that the total wave function, which is an antisymmetrized product of the molecular orbitals (the ϕ_j), remains unchanged for any orthonormal transformation of the ϕ_j and especially on formation of linear sums such as, for example,

$$\begin{vmatrix} \phi_1(1) & \phi_2(1) \\ \phi_1(2) & \phi_2(2) \end{vmatrix} \equiv \begin{vmatrix} d_1(1) & d_2(1) \\ d_1(2) & d_2(2) \end{vmatrix},$$
$$d_{1,2} = (\phi_1 \pm \phi_2)/N^2,$$

where N is a normalizing factor. Thus, for example, the molecules can be described equally well in terms of either molecular orbitals or "equivalent orbitals" localized in a limited part of the molecule. The electronic charge density at a given point depends on the squared modulus of the total wave function, which is the same in both cases.

Hall (17) proposed a semiempirical (and self-consistent) method of treating saturated compounds, based on group orbitals and using experimental ionization potentials to derive the necessary parameters.

Brown (3), starting from somewhat different principles, has put forward a method very similar to that of Hall. Because of their very nature these methods do not make possible the evaluation of electronic charge distribution (except for ions) or of the spectral properties.

We attempted to obtain at least electronic charge distributions by the semi-empirical LCAO MO method. We tried three different approximations.

III. THE "C" APPROXIMATION

Daudel and the author (38) described this method which is only a tentative one because of the rather unjustified approximations involved in it. It has proved to be useful, however, in increasing our practical knowledge concerning computations on saturated compounds.

Let us consider a paraffinic hydrocarbon. If we suppose tetrahedral hybrida-tion we have four sp^3 hybrids on each carbon atom and the $1s$ orbitals of the hydrogen atoms. In this first approximation we not only disregard the hydro-gens but also those hybrids which are linked to the hydrogens. There is no reason to do this but it would be interesting to investigate whether or not con-sideration of the carbon–carbon linkages alone could explain certain properties of these compounds. We put all the coulombic terms equal to $\alpha_C = \alpha = 0$, we neglect interactions between nonadjacent carbon atoms or orbitals not linked together and overlap integrals. (These latter will be taken into account in approximation "H".) Furthermore, for two carbon atoms bound together, we put $\beta_{C-C} = \beta$. Lacking any information concerning interactions between hybrids on the same carbon atom we varied this parameter $\beta' = m\beta$ in certain cases (vide infra).

We define as "orbital charge" the quantity

[II] $$q_r = \sum c_r^2,$$

r being an index for the atomic orbitals, the summation being extended over all molecular spin-orbitals occupied by electrons in the ground state.[*] For n-paraffins we obtain $q_r = 1$ in this approximation using any value of β'. If we write the secular determinant with the above conventions, and if $\beta' = \frac{1}{4}\beta$, we obtain for n-propane the secular determinant given in Table I, where we

TABLE I

C APPROXIMATION. SECULAR DETERMINANT FOR n-PROPANE

	1	2	3	4
1	y	1	0	0
2	1	y	$\frac{1}{4}$	0
3	0	$\frac{1}{4}$	y	1
4	0	0	1	y

have divided everywhere by β and put $|(\alpha - \epsilon)/\beta| = y$, ϵ being the energy corresponding to the molecular orbitals. The system of numbering shown in Fig. 1 was used. This secular equation would be the same as the one for

[*] All atomic and molecular orbitals are normalized to unity. Thus q_r is expressed in electron-units. "Effective" charges are obtained by subtracting from these values the corresponding positive charges in the nuclei.

butadiene in the π-electron approximation if we had put $\beta' = \beta$. Such analogies may be useful in many cases. In the case of certain branched chain hydrocarbons, the secular equation may be analogous to the one of an odd-membered ring conjugated hydrocarbon and thus we might expect to obtain orbital charges different from unity (10).
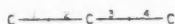
$$C \underline{\quad \quad} C \underline{\quad 2 \quad 4} C$$

FIG. 1. Numbering system for Table I.

Now we replace one CH_3 group in propane by substituent X, and we suppose that $\beta_{X-C} = \beta_{X-X}$, $\alpha_X = \alpha_C + \beta$, and that no other electron of X but the one bound to the next carbon atom has to be considered. Then X will be an imaginary substituent with an electronegativity higher than carbon.

We carry through the calculation successively with $m = +2, +1, +0.25, 0, -0.25, -1,$ and -2 ($\beta' = m\beta$). The result found is that the sign of m does not change the values of the charges in this particular case. The diagrams obtained are shown in Fig. 2. We also computed "n-X-propane" with

FIG. 2. "C" approximation. Orbital charges of "X-ethane", "n-X-propane", and "X-isopropane".
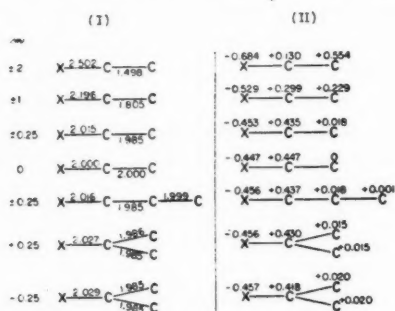
FIG. 3. "C" approximation. (I) Bond charges; (II) Effective atom charges of "X-ethane", "n-X-propane", and "X-isopropane".

$m = \pm 0.25$, which seem to be the most reasonable values. In the case of "X-isopropane" the result is no longer independent of the sign of m. However the results obtained with $m = +0.25$ and $m = -0.25$ lie close to each other.

Other ways of looking at these diagrams are as follows: We may add the orbital charges on one X—C or C—C bond and call them bond charges or we may add the orbital charges on orbitals belonging to one given atom and call them atom charges. (See Fig. 3.) The main conclusions from these diagrams are that, at least in this rough approximation, the electron attraction of the substituent X mainly affects the carbon orbital which contributes to the bond X—C, and that this effect decreases rapidly with increasing distance from X. The orbital charges are alternant as in a conjugated chain but this alternance is masked as soon as we compute the bond changes. There are two orbitals now on each atom instead of one as in pure π-electron calculations.

IV. THE "CH" APPROXIMATION

We here include the hydrogens, although not yet explicitly. The tetrahedral hybrids which bind together two carbons (or one carbon and one X) are taken individually but the hybrids linked to hydrogens are considered as forming a CH group orbital with the $1s$ electron of hydrogen.

This way of setting up the secular equation may be considered, we think, as a compromise between Hall's method of two-atom-orbitals and the straight LCAO treatment, with all the orbitals individualized, as we have attempted in Section V.

In Fig. 4 there are diagrams of n-propane, n-butane, n-pentane, and 2-methyl butane. For computing them we took all the coulombic terms to be equal. We furthermore took $\beta_{C-C} = \beta$ for carbon—carbon bonds, $\beta' = \frac{1}{4}\beta$ for
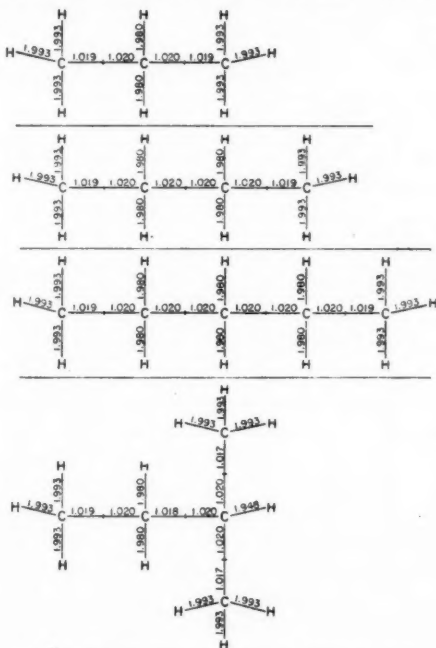
FIG. 4. "CH" approximation. Orbital charges of n-propane, n-butane, n-pentane, and 2-methyl butane.
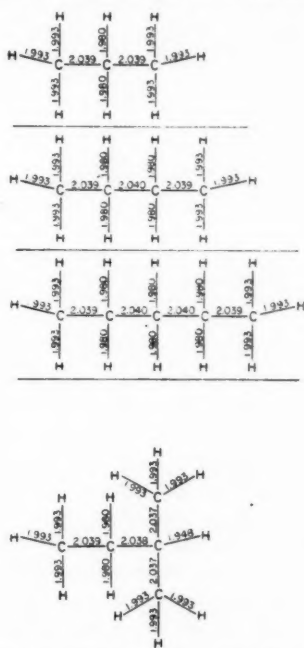
FIG. 5. "CH" approximation. Bond charges of n-propane, n-butane, n-pentane, and 2-methyl butane.

interactions between two tetrahedral hybrids on the same carbon, and also $\beta'' = \frac{1}{4}\beta = \beta'$ for interactions between two C—H bonds on the same carbon. For an interaction between one hybrid and one C—H bond on the same carbon we took $\beta'' = \frac{1}{8}\beta$.

The value 0.25 for β' was chosen according to the preceding approximation and the ratio β'/β'' is about the same as e/d in Lennard-Jones and Hall's (21) treatment of paraffins in their semiempirical method where the choice of

parameters is based on ionization potentials. e is the nondiagonal term between two C—C bonds with a common carbon atom, and d the one between a C—C and a C—H bond also having a carbon atom in common. The numerical values given by these authors were $e = 1.46$ ev. and $d = 0.70$ ev. We suppose here that the interaction between two C—C bonds, with a common carbon atom, may be represented by that of the two hybrids on the central carbon. Concerning the parameter representing the interaction between two C—H bonds on the same carbon the value given by Hall (17) was $b = 1.75$ ev. in the case of methane. Seeking only the right order of magnitude we adopted β' for the corresponding parameter.

The (unfactorized) secular determinant for n-propane is given in Table II. The numbering system on Fig. 6 was used. The diagrams in Fig. 4 give the orbital charges for four hydrocarbons (these are actually bond charges for the CH bonds).
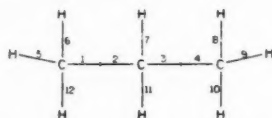
FIG. 6. Numbering system for Table II.

TABLE II

"CH" APPROXIMATION. SECULAR DETERMINANT FOR n-PROPANE

	1	2	3	4	5	6	7	8	9	10	11	12
1	y	1	0	0	¼	¼	0	0	0	0	0	¼
2	1	y	¼	0	0	0	¼	0	0	0	¼	0
3	0	¼	y	1	0	0	¼	0	0	0	¼	0
4	0	0	1	y	0	0	0	¼	¼	¼	0	0
5	¼	0	0	0	y	¼	0	0	0	0	0	¼
6	¼	0	0	0	¼	y	0	0	0	0	0	¼
7	0	¼	¼	0	0	0	y	0	0	0	¼	0
8	0	0	0	¼	0	0	0	y	¼	¼	0	0
9	0	0	0	¼	0	0	0	¼	y	¼	0	0
10	0	0	0	¼	0	0	0	¼	¼	y	0	0
11	0	¼	¼	0	0	0	¼	0	0	0	y	0
12	¼	0	0	0	¼	¼	0	0	0	0	0	y

The first observation is that although we used molecular orbitals in these calculations the bonds appear to be very well "localized". A certain displacement of the electrons takes place from the C—H bonds towards the C—C bonds and the carbon chain thus becomes negatively charged and the C—H bonds positively charged.

Other ways of looking at these results can be seen in the diagrams of "bond charges" (Fig. 5) and "group charges". The latter mean the sum of orbital charges (or CH bond charges) of orbitals (or CH bonds) around a given atom. Effective group charges are 0 for n-paraffins in this approximation. The same numbers occur in all these diagrams (at least with an accuracy of 0.001) and there does not seem to be any alternance in the chain. CH bonds on primary carbon atoms have an effective charge of $+0.007$, CH bonds on secondary carbon atoms have $+0.020$, and CH bonds on tertiary carbon atoms have $+0.052$. This may be related to the observation that nucleophilic sub-

stitution takes place, in order of preference, on $\geqslant$CH, $>$CH$_2$, and —CH$_3$ groups.

If we substitute X in n-butane and n-pentane we obtain the results given in Figs. 7 and 8 (X has the same properties as in Section III).

According to these diagrams almost all the charge pulled on the heteroatom because of its electronegativity being greater than carbon comes from the carbon orbital bonded to it and also from the C—H bonds on that carbon. There still is some perturbation in the next C—C bond but after the second carbon the effect of substituent X becomes negligible and the values of charges are the same as in the related hydrocarbons.
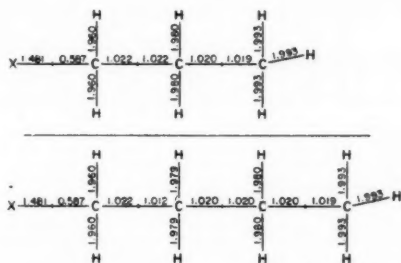
FIG. 7. "CH" approximation. Orbital charges of "n-X-propane" and "n-X-butane".
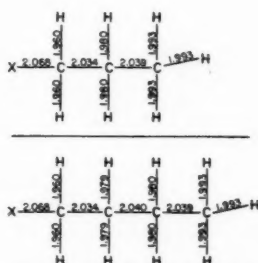
FIG. 8. "CH" approximation. Bond charges of "n-X-propane" and "n-X-butane".

If we compare these diagrams to the one computed by Gouarné and Yvan (16) (Fig. 9) with comparable parameters for a conjugated chain substituted at carbon one, then we see the significant difference between conjugated and saturated chains. The effect of X reaches much further in conjugated compounds. This result is obtained in spite of the use of similar wave functions and shows that, because of the different arrangement of orbitals, certain electrons appear to be "mobile" and others "localized". This also illustrates the fact that the use of molecular orbitals is just a way of writing wave functions and does not necessarily involve the "delocalization" of the electrons.
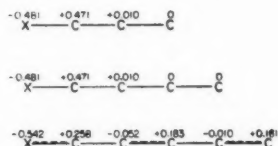
FIG. 9. Comparison between saturated and conjugated compounds. (The diagram of the conjugated compound from Gouarné and Yvan.) Effective atom charges. "CH" approximation.

As the coulombic terms of the C—H bonds are likely to be different from those of the orbitals forming C—C bonds we computed n-X-butane with $\alpha_{C-H} = \alpha_C + \beta$ and $\alpha_{C-H} = \alpha_C - \beta$ (Fig. 10). There seems to be a slight alter-

nance of more and less negative charges in the carbon chain with these para-
meters (in the third decimal), but the main picture remains the same.

When we replace X by a "real" substituent we have to face new difficulties.
Outside of the need for new parameters one has to find a way of including lone
pairs of electrons in semiempirical calculations. Actually, all the usual sub-
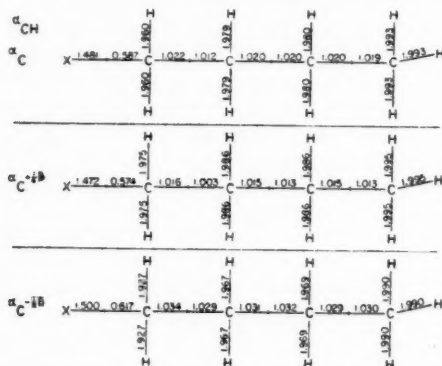stituents of organic compounds contain lone pairs of electrons.

FIG. 10. "CH" approximation. Variation of α_{C-H}. Orbital charges of "n-X-butane".

We only attempted to compute a diagram for 1-amino-propane (Fig. 11).

As Pitzer (32) gave good reasons to believe that C—N bonds are weaker
than C—C bonds (66 kcal. against 80 kcal.) owing to electronic repulsion, we
did not include the electrons of the lone pair directly into the calculation but
gave to β_{C-N} a value equal to $0.8\beta_{C-C}$, to represent the relative weakness of
the C—N bond. Another parameter used was $\alpha_N = \alpha_C + 0.65\beta$ for the coulom-
bic term of nitrogen (supplement equal to the difference of electronegativities
of nitrogen and carbon). Interaction between the two N—H bonds was con-
sidered to be like that between two C—H bonds and interaction between
H—N and N—C to be like that between H—C and C—C.

Fig. 11 needs little comment.
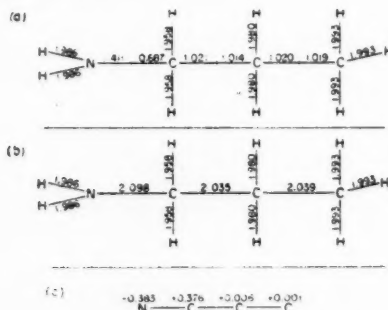
FIG. 11. "CH" approximation. 1-Amino-propane: (a) orbital charges, (b) bond charges,
(c) effective atom charges.

V. THE "H" APPROXIMATION

In our third approximation all sp^3 hybrids and also all hydrogen $1s$ orbitals are included separately.

This has never been done before, to our knowledge, in a semiempirical treatment. It is not easy to select the parameters. We included overlap integrals right from the beginning. Overlap integrals are very high in σ-bonds. In spite of this we did not introduce them in approximations "C" and "C—H". We did not because, as Chirgwin and Coulson (4) have shown, this does not alter the values of charges provided all the coulombic terms are equal, and thus it does not affect our calculations related to pure hydrocarbons. It does change the charges in the case of the substituted compounds. It is unlikely however to change the qualitative features. Overlap integrals are fully taken into account in approximation "H".

Mulliken, Rieke, Orloff, and Orloff (29) computed overlap integrals using Slater functions. From their tables we obtain:

$$S_{C-H} = 0.684,$$
$$S_{C-C} = 0.647,$$
$$S_{intra} = -0.500.$$

S_{intra} is the overlap integral between two sp^3 hybrids on the same carbon atom. We put the β's proportional to the overlap integrals and the coulombic integrals equal to the electronegativity of the atoms minus the electronegativity of carbon (C: 2.50; H: 2.00).

Using Wheland's (40, 41) approximation the coulombic term corresponding to substituent X becomes

$$y + \delta_X(1 - Sy) = 0.35y + 1,$$

with $\delta_X = 1$ and $S = 0.65$.

For a hydrogen atom

$$y + \delta_H(1 - Sy) = y - 0.50(1 - 0.65y) = 1.33y - 0.50$$

FIG. 12. Numbering system for Table III.

with $\delta_H = -0.50$. The nondiagonal terms become, for an interaction between two adjacent carbon atoms (resonance integrals including overlap), $\gamma_{C-C} = 1$; between X and C, $\gamma_{X-C} = 1$; between C and H, $\gamma_{C-H} = 0.684/0.647 = 1.06$; between two hybrids on the same carbon, $\gamma_{intra} = -0.500/0.684 = -0.77$. With these values the secular determinant for "n-X-propane" (Fig. 12) becomes as shown in Table III.

TABLE III

"H" APPROXIMATION. SECULAR DETERMINANT FOR "n-X-PROPANE"

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	$(0.33y+1)$	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	y	-0.77	-0.77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	-0.77	y	-0.77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.06
4	0	-0.77	-0.77	y	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	1	y	-0.77	-0.77	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	-0.77	y	-0.77	0	0	0	0	0	0	0	0	0	0	0	1.06	0
7	0	0	0	0	-0.77	-0.77	y	1	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	1	y	-0.77	-0.77	-0.77	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	-0.77	y	-0.77	-0.77	0	0	0	0	0	0	1.06	0	0
10	0	0	0	0	0	0	0	-0.77	-0.77	y	-0.77	0	0	0	0	0	1.06	0	0	0
11	0	0	0	0	0	0	0	-0.77	-0.77	-0.77	y	0	0	0	0	1.06	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	y	0	0	1.06	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	y	1.06	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	1.06	$(1.33y-0.5)$	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	1.06	0	0	$(1.33y-0.5)$	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	1.06	0	0	0	0	$(1.33y-0.5)$	0	0	0	0
17	0	0	0	0	0	0	0	0	0	1.06	0	0	0	0	0	0	$(1.33y-0.5)$	0	0	0
18	0	0	0	0	0	0	0	0	1.06	0	0	0	0	0	0	0	0	$(1.33y-0.5)$	0	0
19	0	0	0	0	0	1.06	0	0	0	0	0	0	0	0	0	0	0	0	$(1.33y-0.5)$	0
20	0	0	1.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$(1.33y-0.5)$

FIG. 13. "H" approximation. n-propane (see text).
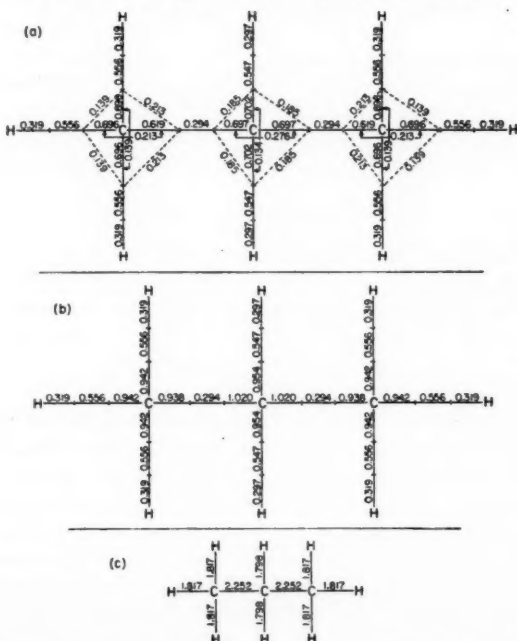
FIG. 14. "H" approximation. $(+)$-Ion of n-propane (see text).
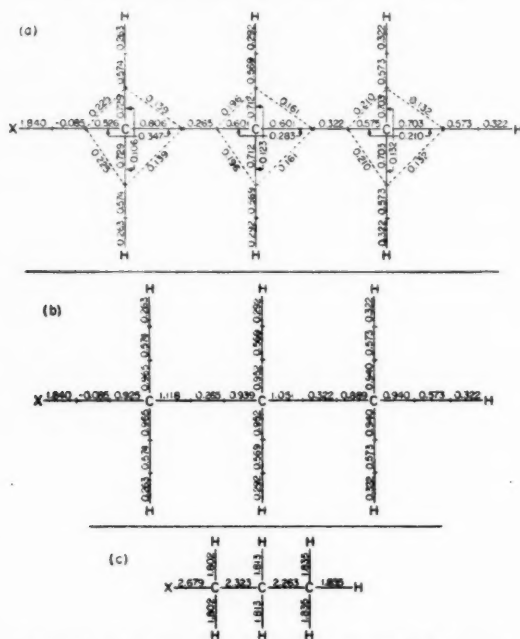
FIG. 15. "H" approximation. "n-X-propane" (see text).
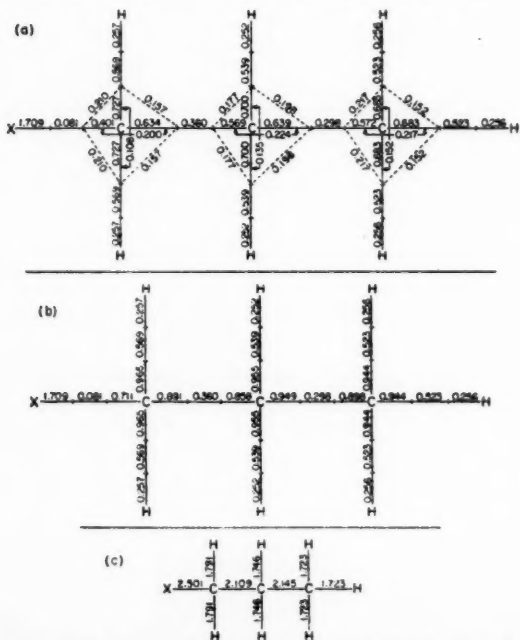
FIG. 16. "H" approximation. $(+)$-Ion of "n-X-propane" (see text).

In Figs. 13–14 we find diagrams for n-propane and its $(+)$ ion, which we obtain by taking away an electron from the molecular orbital of highest energy. In Figs. 15 and 16 are diagrams for "n-X-propane" and its $(+)$ ion. (Length of lines has no quantitative meaning in these figures.)

It has been long recognized that inclusion of overlap integrals makes definition of charges (and bond orders) more delicate (4, 23).

The most natural way of representing charge distribution in our opinion is the one suggested by MacWeeny (24) for conjugated compounds. He defines *local* charges as

[III] $$q_r = \sum c_r{}^2$$

and

[IV] $$q_{rs} = S_{rs} \sum c_r c_s.$$

In this manner we obtain diagrams (b) in Figs. 13–16, which are comparable with MacWeeny's diagram of naphthalene.

By adding all the charges in each chemical bond we obtain diagrams (c).

The coulombic terms we used are very probably too high and effective charges are lower in reality. This does not however affect our conclusions.

In C—H bonds the high value of overlap charge shows the trend of the electrons to approach nearer to the carbon. Again the C—C bonds have effective negative charges and the C—H bonds positive ones. If we compare the diagrams of n-propane with those of its $(+)$ ion we find that the electronic charge is mainly taken away from the hydrogens. This is in accord with their lower electronegativity.

Now if we compare n-propane to "X-propane" we see that by far the greatest perturbation takes place in the X—C bond. (Effective charges are relatively higher in this approximation.) In the $(+)$ ion of X-propane the relatively greatest share of the missing electron is taken away from the carbon next to the electronegative substituent X.

VI. CONCLUSIONS

It is believed, on the basis of the above results, that the LCAO MO method, with some refinements, will be capable of giving as good an account of electronic distribution in saturated hydrocarbons and their derivatives as in the case of conjugated compounds.

RÉSUMÉ

La simple méthode des orbitales moléculaires a été appliquée en trois approximations différentes à l'évaluation des diagrammes moléculaires des hydrocarbures saturés. Ces diagrammes qui donnent une idée de la répartition des électrons dans ces molécules permettent d'interpréter quelques différences fondamentales entre corps saturés et corps non-saturés à insaturation conjuguée. (Localisabilité des liaisons, l'effet d'un substituant électronégatif.)

Pour la première fois les électrons $1s$ des hydrogènes ont été introduits dans un calcul à l'aide de la méthode semiempirique des orbitales moléculaires.

ACKNOWLEDGMENTS

REFERENCES

1. ALTMANN, S. L. Proc. Roy. Soc. (London), A, 210: 327. 1951.
2. ARTMANN, K. Z. Naturforsch. 1: 426. 1946.
3. BROWN, R. D. J. Chem. Soc. 2615. 1953.
4. CHIRGWIN, B. H. and COULSON, C. A. Proc. Roy. Soc. (London), A, 201: 196. 1950.
5. COULSON, C. A. Proc. Phys. Soc. (London), 60: 257. 1948.
6. COULSON, C. A. and FISCHER, I. Phil. Mag. 40: 386. 1949.
7. COULSON, C. A. and LONGUET-HIGGINS, H. C. Proc. Roy. Soc. (London), A, 191: 39. 1947.
8. COULSON, C. A., MARCH, N. H., and ALTMANN, S. L. Proc. Natl. Acad. Sci. U.S. 38: 372. 1952.
9. CRAIG, D. P. Proc. Roy. Soc. (London), A, 200: 474. 1950.
10. DAUDEL, R. Private communication.
11. DAUDEL, R., ODIOT, S., and BRION, H. J. chim. phys. 51: 74, 358, 361, 554. 1954.
12. DAUDEL, R., SANDORFY, C., VROELANT, C., YVAN, P., and CHALVET, O. Bull. soc. chim. France, 17: 66. 1949.
13. DEWAR, M. J. S. The electronic theory of organic chemistry. The Clarendon Press, Oxford. 1949.
14. DEWAR, M. J. S. and LONGUET-HIGGINS, H. C. Proc. Roy. Soc. (London), A, 214: 482. 1952.
15. GOEPPERT-MAYER, M. and SKLAR, A. L. J. Chem. Phys. 6: 645. 1938.
16. GOUARNÉ, R. and YVAN, P. Compt. rend. 228: 1345. 1949.
17. HALL, G. G. Proc. Roy. Soc. (London), A, 205: 541. 1951.
18. HÜCKEL, E. Z. Physik, 70: 204. 1931.
19. LENNARD-JONES, J. E. Trans. Faraday Soc. 25: 668. 1929.
20. LENNARD-JONES, SIR J. Proc. Roy. Soc. (London), A, 198: 1, 14. 1949.
21. LENNARD-JONES, SIR J. and HALL, G. G. Trans. Faraday Soc. 48: 355. 1952.
22. LINNETT, J. W. and POË, A. J. Trans. Faraday Soc. 47: 1033. 1951.
23. LÖWDIN, P.-O. J. Chem. Phys. 18: 365. 1950.
24. MACWEENY, R. J. Chem. Phys. 19: 1614. 1951.
25. MOSER, C. M. J. Chem. Phys. 21: 2098. 1953.
26. MULLIKEN, R. S. Phys. Rev. 41: 49. 1932.
27. MULLIKEN, R. S. J. Chem. Phys. 7: 14, 20, 121, 339, 353. 1939.
28. MULLIKEN, R. S. J. chim. phys. 46: 497. 1949.
29. MULLIKEN, R. S., RIEKE, C. A., ORLOFF, D., and ORLOFF, H. J. Chem. Phys. 17: 1248. 1949.
30. PAULING, L. The nature of the chemical bond. Cornell Univ. Press, Ithaca, N.Y. 1945.
31. PAULING, L. and WILSON, E. B. Introduction to quantum mechanics. McGraw-Hill Book Company, Inc., New York and London. 1935.

32. PITZER, K. S.   J. Am. Chem. Soc. 70: 2140.   1948.
33. PLATT, J. R.   J. Chem. Phys. 18: 1168.   1950.
34. PULLMAN, A. and PULLMAN, B.   Les théories électroniques de la chimie organique.   Masson et Cie, Paris.   1952.
35. ROGER, F.   Compt. rend. 236: 2207.   1953.
36. ROOTHAAN, C. C. J.   Revs. Mod. Phys. 23: 69.   1951.
37. Ross, I. G.   Trans. Faraday Soc. 48: 973.   1952.
38. SANDORFY, C. and DAUDEL, R.   Compt. rend. 238: 93.   1954.
39. VROELANT, C.   Compt. rend. 236: 1666, 1887.   1953.
40. WHELAND, G. W.   J. Am. Chem. Soc. 63: 2025.   1941.
41. WHELAND, G. W.   J. Am. Chem. Soc. 64: 900.   1942.
42. WHELAND, G. W. and PAULING, L.   J. Am. Chem. Soc. 57: 2086.   1935.
43. ZIMMERMANN, H. K. and VAN RYSSELBERGHE, P.   J. Chem. Phys. 17: 598.   1949.

STUDIES ON THE HETEROGENEITY OF CARRAGEENIN[1]

BY DAVID B. SMITH,[2] A. N. O'NEILL,[3] AND A. S. PERLIN[2,4]

ABSTRACT

The carbohydrate residues in κ-carrageenin are 3,6-anhydro-D-galactose and D-galactose which are present in nearly equal amounts. Tests following fractional precipitation of κ-carrageenin indicate general chemical homogeneity but physical heterogeneity. κ-Carrageenin is not susceptible to oxidation with periodate. Fractional precipitation of λ-carrageenin separates a main fraction which contains only D-galactose and a trace of 3,6-anhydro-D-galactose. This polysaccharide is susceptible to periodate oxidation. It is polydisperse on a mass basis. Materials containing glucose, xylose, and L-galactose segregate into minor fractions of λ-carrageenin and hence cannot be integral parts of the principal polysaccharides of carrageenin. L-Galactose has been isolated from carrageenin by a simple procedure.

Carrageenin is the polysaccharide complex which is extracted with water from certain red seaweeds, in particular, *Chondrus crispus* and *Gigartina stellata* (26). D-Galactose comprises about two-thirds of the organic matter. The principal remaining organic constituent is 3,6-anhydro-D-galactose recently identified by O'Neill (17, 18). The presence of small amounts of glucose, pentose, and L-galactose has been established (2, 13). Carrageenin contains also about 30% of monoesterified sulphuric acid and consequently, in neutral solution, exists as a salt.

Chemical evidence (4, 13, 15) bearing on the structure of carrageenin has usually been interpreted on the basis of a monotypical substance though the possibility of greater complexity has been admitted (13). Differences in degree of gel formation and other properties among fractions obtained by extraction at successively higher temperatures (7, 9, 10, 20) have been taken to indicate different polysaccharides. However, the principal chemical difference reported in the earlier literature has been in cation ratios and it has been suggested that the polysaccharides are otherwise essentially identical (2).

The development of a new method of fractionation (23) based on the gelatinizing effect of potassium ions on carrageenin has opened a new approach to the structure of this material. This fractionation, provided it is performed on sufficiently dilute carrageenin solutions, permits a sharp separation into potassium-sensitive material which is precipitated by potassium ions and potassium-insensitive material which remains in solution (23, 24).

This paper describes some analytical and physical characterization of subfractions of these two materials. The suggestion made previously (24) that the potassium-sensitive fraction (κ-carrageenin) would be more chemically homogeneous than the potassium-insensitive material (λ-carrageenin) is confirmed.

MATERIALS AND METHODS

Preparation of Carrageenin and Its κ- and λ-Components

Dried *Chondrus crispus* was ground coarsely, washed briefly with cold water, and extracted in 1% suspension for one hour at 90°C. in water containing 0.2% NaCl and 0.1% Na₂CO₃. Insoluble material was filtered out and re-extracted for one hour at 90°C. using one-third the first volume of extractant. The combined extracts were dialyzed against several changes of 0.2 M NaCl to remove ions other than Na⁺. The dialyzed solution was clarified by centrifugation at 2000 g. The carrageenin was precipitated from a portion of this solution with three volumes 95% ethanol and was washed and dried with ethanol and ether. This sample was designated CL3.

The remaining carrageenin solution was diluted with water to 0.24% solids. Solid KCl was added slowly with stirring to a concentration of 0.25 M. The crude κ-carrageenin precipitate was separated by centrifugation at 2000 g. The precipitate was dialyzed first against 0.1 M NaCl to remove potassium ions and then against water to remove NaCl. The κ-carrageenin dissolved and was diluted to about 0.2%. Potassium chloride was added to 0.25 M and the κ-carrageenin again separated by centrifugation. Dialysis with solution of the κ-carrageenin and its reprecipitation were repeated once more. The final κ-carrageenin precipitate was suspended in 0.5 M NaCl, dialyzed free of potassium ions, and precipitated with three volumes of ethanol. After the precipitate was washed free of chloride ion with 80% ethanol followed by absolute ethanol and ether, it was dried over CaCl₂. This sodium κ-carrageenate preparation was designated CL3 κ-1.

The supernatants from the foregoing centrifugations (with the exception of the last which contained a negligible amount of polysaccharide) were combined and evaporated at 35°C. to one-tenth the initial volume. The λ-carrageenin (CL3 λ-1) was precipitated from this solution, and washed and dried in the same manner as the previous preparations.

Fractionation of κ- and λ-Carrageenins

κ-Carrageenin (CL3 κ-1) was fractionated by precipitation from dilute solution (0.2% in 0.25 M NaCl) by the graded addition of ethanol at 20–25°C. The precipitate was removed after each addition of ethanol by centrifugation. The fractions were washed free of chloride ion and dried over CaCl₂ after absolute ethanol and ether washes. The ethanol concentration ranges used for fractionation and the yields obtained are shown in Table I. (Considerable loss of material, particularly of Fraction 2a, attended the first fractionation.)

The fractionation of λ-carrageenin (preparation C5 λ-2) has been described before (24). Particulars and yields are reproduced, for reference, in Table I.

Physical Measurements

Viscosity at 25°C. and sedimentation rates were measured in the manner already described (24). For extrapolation of the sedimentation results to zero concentration the method of Newman, Loeb, and Conrad (16) was used.

TABLE I

SUBFRACTIONATION OF CARRAGEENIN FRACTIONS

Fractionation No.	Fraction obtained	Ethanol concentration range, %	Yield (% of original sample)
1	CL3 κ-1 Fr. 1a	0–30	2
	Fr. 2a	30–45	35
	Fr. 3a	45–75	16
2	CL3 κ-1 Fr. 1b	0–35	4
	Fr. 2b	35–45	64
	Fr. 3b	45–75	25
3	C5 λ-2 Fr. 1	0–12	1.4
	Fr. 2	12–24	2
	Fr. 3	24–36	62
	Fr. 4	36–48	11
	Fr. 5	48–60	1.4

Analytical Methods

Sulphate was determined after refluxing for 24 hr. in 0.5 N HCl followed, after tenfold dilution, by precipitation as $BaSO_4$.

For chromatographic analyses, samples (1% in 0.5 N H_2SO_4) were hydro-lyzed in sealed tubes for 10–15 hr. on a boiling water bath. After neutraliza-tion of the hydrolyzates with $BaCO_3$ and filtration, the filtrates were examined by descending paper chromatography (12, 19). For quantitative chromato-graphic analysis, the method of Flood et al. (5) was used. Isolation of galactose as the crystalline α-methyl-α-phenylhydrazone was also employed for galactose estimation.

L-Galactose was taken to be the galactose remaining after treatment of the neutral hydrolyzate with a suspension of a galactose fermenting yeast, Saccharomyces cerevisae, strain N.R.C. No. 824, (16 hr. culture from D-galactose agar) at 37°C. for 3.5 hr. The activity of the culture was simultaneously tested with a 1% solution of D-galactose. After incubation, the yeast, together with added charcoal, was separated by centrifugation and the supernatant liquor examined on the chromatogram. D-Galactose was completely fermented as was glucose, but xylose was unaffected.

3,6-Anhydro-D-galactose cannot be determined directly because of its instability during acid hydrolysis. The following procedure takes advantage of its degradation under such conditions to 5-hydroxymethyl-2-furaldehyde which can be determined spectrophotometrically. Samples (25 mgm.) were weighed into tubes containing 2 ml. 0.15 N H_2SO_4. After they were sealed, the tubes were placed in a bath at 100°C. At intervals tubes were removed, their contents neutralized with $BaCO_3$ and filtered quantitatively into 10 ml. volu-metric flasks which were made to volume. The optical densities at 2850 Å (the characteristic absorption maximum for 5-hydroxymethyl-2-furaldehyde) were determined on suitable dilutions. Since 5-hydroxymethyl-2-furaldehyde undergoes a first order decomposition under the above conditions to formic and levulinic acids (25), the logarithms of the optical densities were plotted against time and the curves extrapolated to zero time (Fig. 1). From these extrapolated values and the molar extinction coefficient (16,500) of 5-hydroxy-methyl-2-furaldehyde, the initial amounts of the latter were estimated and recalculated as 3,6-anhydro-D-galactose.
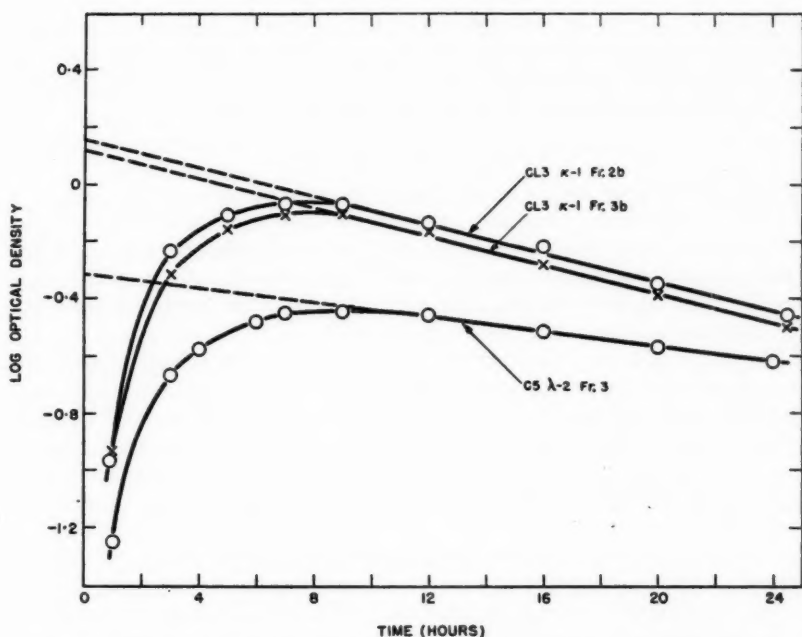
FIG. 1. Course of the formation and disappearance of 5-hydroxymethyl-2-furaldehyde.

Isolation and Identification of L-Galactose

Carrageenin (sample C5 (24), 8 gm.) was hydrolyzed in 800 ml. 0.3 N H_2SO_4 for four hours at 100°C. The hydrolyzate was neutralized with $BaCO_3$, decolorized with charcoal, and incubated at 37°C. for four hours with a suspension of *S. cerevisae* cells (strain N.R.C. No. 824) from a 16 hr. culture in D-galactose broth. After the cells were precipitated with charcoal and the solution concentrated, the sirup (1.3 gm.) was chromatographed on a cellulose column using *n*-butanol saturated with water as the developing solvent (11). Those portions of the eluate containing galactose were combined and dried *in vacuo* at 50°C. The residue (0.11 gm.) was extracted with water and the extract was incubated with yeast cells, as above. The product recovered from this second fermentation by extraction into alcohol readily crystallized in the cold. Amount obtained was 0.09 gm., m.p. 162–163°C. Two recrystallizations raised the melting point to 167°C. The X-ray powder diagram was identical with that of D-galactose, but the optical rotation was negative, $[\alpha]_D^{27}$ $-96°$ (15 min.) $\rightarrow$ $-76°$ (eqm.) (*c* 2.0, water). This product was, therefore, α-L-galactose.

Oxidation with Periodate

Samples (100 mgm.) of carrageenin fractions were dissolved in 25 ml. water (solution was neutral to methyl red) at 16.6°C. and 250 mgm. sodium metaperiodate was added. Formic acid was estimated on aliquots removed from

the reaction mixture at intervals by titration in the presence of potassium iodide with thiosulphate after the addition of excess ethylene glycol. Periodate consumption was measured iodometrically on similar aliquots after the addition of sodium bicarbonate, arsenite, and potassium iodide (8).

RESULTS AND DISCUSSION

Paper chromatograms of hydrolyzates of κ-carrageenin showed only two principal components, identified as galactose and 5-hydroxymethyl-2-furaldehyde. The latter has been shown to be a decomposition product of 3,6-anhydro-D-galactose which can be isolated from κ-carrageenin as the crystalline diethylmercaptal obtained after mercaptolysis of the polysaccharide (17, 18). A trace of xylose could also be detected.

Chromatography of hydrolyzates of λ-carrageenin revealed galactose as the main component, lesser amounts of glucose and xylose, and only a trace of 5-hydroxymethyl-2-furaldehyde. Examination of the fractions of λ-carrageenin indicated that Fraction 3 contained predominantly galactose with a trace of 5-hydroxymethyl-2-furaldehyde, that Fraction 4 contained mostly galactose but also glucose and xylose, and that Fraction 5 also contained these three sugars but that galactose had become a minor constituent. Fraction 5 gave a red color with iodine. These results suggest that xylose and glucose polymers are not integral components of carrageenin but represent small amounts of xylan and floridean starch coextracted by the isolation procedure. From this point of view, the trace of xylose found in κ-carrageenin could also be ascribed to contamination.

A small proportion of the galactose residues in carrageenin was reported by Johnson and Percival (13) to consist of L-galactose. In the present study, however, it became evident that the component containing L-galactose was separable by fractional precipitation from the D-galactose polymers which comprise the bulk of carrageenin. After fermentation with a yeast strain capable of utilizing D-galactose, a reference solution of D-galactose and a hydrolyzate of κ-carrageenin were found to contain no galactose, a hydrolyzate of C5 λ-2 Fraction 3 showed only a trace of galactose, but hydrolyzates of C5 λ-2 Fractions 4 and 5 gave strong tests for galactose. The "tail" fractions of λ-carrageenin thus account for most of the L-galactose. This sugar therefore is not associated with the polysaccharides which make up the greater part of carrageenin.

The validity of these fermentation tests was assessed by the isolation of L-galactose from the nonfermented residue of a large-scale fermentation. Although the yield of L-galactose was low (about 2% of the sugars present in carrageenin), the availability of the starting material and the ease of isolation of the L-galactose make carrageenin a ready source of this rare sugar. May and Weinland (14) have recently isolated L-galactose from snail galactogen by a similar procedure.

After 220 hr. reaction time, there was no detectable consumption of periodate by κ-carrageenin. This result conforms with the formulation of κ-carrageenin as consisting of D-galactose-4-sulphate residues glycosidically linked through

carbon atoms 1 and 3 (2) and with the presence of 3,6-anhydro-D-galactose residues (17, 18). The possibility of branching (1, 4, 13) is not precluded, but the terminal units of such branches must be 3,6-anhydro-D-galactose since end units of D-galactose-4-sulphate would consume periodate at the 2,3-glycol configuration. The lack of effect of periodate on κ-carrageenin is in agreement with the previous finding (23) that after treatment of whole carrageenin with sodium periodate, the κ-fraction could still be precipitated by potassium chloride.

In a parallel experiment, the main fraction of λ-carrageenin (C5 λ-2 Fr. 3) consumed, per mole of sugar residue, 0.30 moles of periodate in 165 hr. and 0.31 moles in 220 hr. At the same reaction times 0.12 and 0.13 moles respectively of formic acid were produced. Since, therefore, one unit in five must have at least one free diol group and one unit in eight, a free triol group, this fraction cannot consist only of a chain of 1,3-linked D-galactose-4-sulphate residues. A model involving branching could account for the periodate consumption with some residues unesterified. It would be necessary that some of the latter be end groups to yield formic acid. Johnson and Percival (13) and Dillon and O'Colla (4) have presented evidence of branching from methylation studies.

The reaction of C5 λ-2 Fraction 3 with periodate raises doubts about the use of this oxidant in the method whereby Dillon and O'Colla (4) "purified" carrageenin. These workers ascribed the consumption of periodate to oxidation of floridean starch and cellulose. Fraction 3 contained neither of these materials.

The results of analyses of some of these carrageenin preparations are given in Table II. Though the sum of the residues found totals only about 90% in

TABLE II
ANALYTICAL RESULTS

Material	Galactose, %	3,6-Anhydro-D-galactose, %	SO$_3$Na, %	Total recovery, %	Molar ratio, galactose / SO$_3$Na
CL3 κ-1	38.1	24.0	28.2	90.3	1.03
CL3 κ-1 Fr. 2b	35.0	28.1	28.1	91.2	0.95
CL3 κ-1 Fr. 3b	—	25.9	28.8	—	—
C5 λ-2 Fr. 3	46.3	1.8	37.9	89.4*	0.90

*Contains 3.4% of contaminating NaCl.

both the κ-carrageenin and the principal fraction of λ-carrageenin, this discrepancy is believed to represent the sum of determination errors since, with the exception of a trace of xylose in the κ-carrageenin preparation, no components other than those listed could be found. The content of 3,6-anhydro-D-galactose is much lower in the principal fraction of λ-carrageenin than in κ-carrageenin while the galactose content is higher. The slightly smaller amount of 3,6-anhydro-D-galactose found in unfractionated κ-carrageenin compared with the mean content of its two major subfractions suggests that the small first fraction may be low in the anhydro sugar. Unfortunately

insufficient amounts of the first fraction were available for analysis. The amount of sulphate nearly parallels the galactose content with molar ratios of galactose to sulphate of about unity with κ-carrageenin and about 0.9 with λ-carrageenin. The sulphate contents recorded in Table II confirm those reported earlier (23).

The results of viscosity and sedimentation rate measurements on carrageenin preparation CL3, its κ-component, and the fractions of the κ-carrageenin are shown in Table III. Also included are molecular weights computed using a random coil model by the Mandelkern–Flory equation and using an ellipsoid model by the Perrin and Simha equations (24).

TABLE III

VISCOSITY, SEDIMENTATION, AND MOLECULAR WEIGHT DETERMINATIONS

| Material | $[\eta]$, dl./gm. | $S_{20}^{\circ} \times 10^{13}$ $c \to 0$ | Mol. wt. $\times 10^{-6}$ | |
			(Mandelkern and Flory)	(Perrin and Simha)
CL3	13.2±0.57	8.3	9.5	5.4
CL3 κ-1	12.6±0.78	7.2	7.5	4.3
CL3 κ-1 Fr. 1a	7.9±0.19	4.7	3.1	1.8
Fr. 2a	11.4±0.29	6.2	5.7	3.3
Fr. 3a	10.8±1.35	7.7	7.7	4.4
Fr. 1b	9.5±0.09	5.9	4.8	2.8
Fr. 2b	10.0±0.30	5.9	5.0	2.9
Fr. 3b	8.5±0.51	7.2	5.3	3.7

If the fractionation of the κ-carrageenin had been based solely on size, $[\eta]$, S, and M should have decreased as the mean alcohol concentration required for the precipitation of the fractions increased. However, this simple pattern, adhered to in the subfractionation of C5 λ-2 Fraction 3 (24), was not found in the fractionation of κ-carrageenin. Hence the differences in solubility responsible for the fractionation must reflect (besides size differences) differences in structure, composition, or both. Since the analytical data (Table II) suggest general similarity of composition, it is likely that the solubility differences may be partly explained by structural differences (varying degrees and types of branching).

CONCLUSIONS

Previous physical investigations of carrageenin and carrageenin extracts obtained at different temperatures by viscosity, sedimentation, diffusion, osmotic, and electrophoretic methods (3, 6, 7, 20, 21) have provided evidence that these materials are heterogeneous on a mass basis. Some of this heterogeneity is due to the differences between κ- and λ-carrageenins. However, both of these components, particularly λ-carrageenin (24), have been shown to be polydisperse.

The differences between κ- and λ-carrageenin in response to potassium and similar ions, in sulphate content, optical rotation (23), effect on milk viscosity (22), shape, and size (24), have a chemical basis in the greatly differing D-galactose: 3,6-anhydro-D-galactose ratios. Bayley (1) has used these compositional data in conjunction with measurements of intramolecular spacings from

X-ray diffraction studies to put forward a tentative explanation of the potassium sensitivity of κ-carrageenin.

Probably many of the differences in behavior between carrageenin extracts obtained at different temperatures are due to differing contents of κ- and λ-carrageenins (23). Other factors must include the average size and nature of the mass polydispersity of these components within the extracts, the chemical heterogeneity, and the cation content.

At the present stage in the investigation of the structure of carrageenin, two major and several minor components can be discerned.

1. One of these major components contains D-galactose and 3,6-anhydro-D-galactose residues in a ratio of approximately 1.4 : 1 together with about 25% esterified sulphate. Since fractionation studies have provided evidence of both mass and structural polydispersity, it is not inconceivable that considerable variation in the relative amounts of these residues may be found if the material is fractionated more narrowly. The greater part (perhaps all) of this component is aggregated or gelled by the addition of potassium ions. Though nearly all the material that may be precipitated from dilute (0.1%) aqueous solutions by potassium chloride is precipitated at 0.15 M, a small additional amount is precipitated between 0.15 M and 0.25 M (24). It may be that small quantities of this component are not readily precipitated by potassium ions and would consequently be left in the supernatant after centrifugation. The small amount of 3,6-anhydro-D-galactose found in C5 λ-2 Fraction 3 (Table II) may have arisen in this way.

The extension of the original definition of κ-carrageenin (23, 24) from the material affected by potassium ions to include all of the component consisting of D-galactose, considerable amounts of 3,6-anhydro-D-galactose, and esterified sulphate is proposed.

2. In the second major component of carrageenin, D-galactose together with about 35% esterified sulphate greatly predominates over the small amount of 3,6-anhydro-D-galactose which may be an impurity. It is suggested that the term λ-carrageenin, hitherto (24) referring to all the material not precipitable by potassium, be restricted to this polysaccharide.

3. Minor amounts of various polysaccharides are found in carrageenin preparations and may be regarded as impurities. On hydrolysis, these give rise to glucose (possibly from floridean starch), xylose (possibly from a xylan), and L-galactose.

The similarities between the two principal types (κ- and λ-carrageenins) suggest that intergrading material may exist. However, in those samples of carrageenin so far tested, the sharp fractionation achieved indicates that the amount of such intermediate material must be small.

REFERENCES

1. BAYLEY, S. T.　Biochim. et Biophys. Acta.　In press.
2. BUCHANAN, J., PERCIVAL, E. E., and PERCIVAL, E. G. V.　J. Chem. Soc. 51.　1943.
3. COOK, W. H., ROSE, R. C., and COLVIN, J. R.　Biochim. et Biophys. Acta, 8: 595.　1952
4. DILLON, T. and O'COLLA, P.　Proc. Roy. Irish Acad. 54: 51.　1951.
5. FLOOD, A. E., HIRST, E. L., and JONES, J. K. N.　J. Chem. Soc. 1679.　1948.
6. GORING, D. A. I.　J. Colloid Sci. 9: 141.　1954.
7. GORING, D. A. I. and YOUNG, E. G.　Can. J. Chem. 33: 480.　1955.
8. GRANGAARD, D. H., GLADDING, E. K., and PURVES, C. B.　Paper Trade J. 115 (No. 7): 41.　1942.
9. HAAS, P. and HILL, T. G.　Ann. Appl. Biol. 7: 352.　1921.
10. HESS, M. P. and SIEHRS, A. E.　U.S. Patent No. 2,462,398.　1949.
11. HOUGH, L., JONES, J. K. N., and WADMAN, W. H.　J. Chem. Soc. 2511.　1949.
12. JERMYN, M. A. and ISHERWOOD, F. A.　Biochem. J. (London), 44: 402.　1949.
13. JOHNSTON, R. and PERCIVAL, E. G. V.　J. Chem. Soc. 1944.　1950.
14. MAY, F. and WEINLAND, H.　Z. physiol. Chem. 296: 154.　1954.
15. MORI, T.　Advances in Carbohydrate Chem. 8: 315.　1953.
16. NEWMAN, S., LOEB, L., and CONRAD, C. M.　J. Polymer Sci. 10: 463.　1953.
17. O'NEILL, A. N.　Abstracts of Papers. 126th meeting, Am. Chem. Soc. Sept. 1954.　p. 9D.
18. O'NEILL, A. N.　J. Am. Chem. Soc.　77: 2837.　1955.
19. PARTRIDGE, S. M.　Nature, 158: 270.　1946.
20. ROSE, R. C.　Can. J. Research, F, 28: 202.　1950.
21. ROSE, R. C. and COOK, W. H.　Can. J. Research, F, 27: 323.　1949.
22. SMITH, D. B.　Can. J. Technol. 31: 209.　1953.
23. SMITH, D. B. and COOK, W. H.　Arch. Biochem. and Biophys. 45: 232.　1953.
24. SMITH, D. B., COOK, W. H., and NEAL, J. L.　Arch. Biochem. and Biophys. 53: 192.　1954.
25. TEUNISSEN, H. P.　Rec. trav. chim. 49: 784.　1930; 50: 1.　1931.
26. TSENG, C. K.　Science, 101: 597.　1945.

SOME NEW REACTIONS AND DERIVATIVES OF KOJIC ACID[1]

By Andrew Beélik[2] and C. B. Purves

ABSTRACT

The stability of the ring structure in kojic acid toward various reagents was investigated. Cleavage to an open-chain, dienol derivative in N sodium hydroxide at 25°, or fragmentation of the structure, was very slow. The benzoyl group in the fifth (phenolic) position of dibenzoylkojic acid was removed by hydroxylamine hydrochloride in pyridine so selectively that the method was of value in synthesizing certain new derivatives. Although catalytic hydrogenation readily reduced the pyrone ring in kojic acid to undefined substances, zinc dust in glacial acetic acid reduced the hydroxymethyl group in the dibenzoate to a methyl group and yielded benzoylallomaltol. The ring in dibenzoylkojic acid was apparently opened, with retention of both benzoyl groups, by semicarbazide hydrochloride and pyridine to yield two isomeric "disemicarbazones", $C_{22}H_{22}N_6O_7$, decomp., 215° and 172–173°, respectively. The higher-melting isomer when boiled with dilute acid gave a compound $C_{21}H_{18}N_3O_5$, m.p. 244°, which was apparently cyclic; the same isomer with nitrous acid yielded two isomeric, apparently open-chain "monosemicarbazones", $C_{21}H_{19}N_3O_7$, decomp., 215° and 178–179°, respectively. The structures of these five compounds were not determined. The following substances were thought to be new: sodium kojate, a white powder, and its crystalline tetrahydrate; 5-hydroxy-2-(methoxymethyl)-γ-pyrone, m.p. 75–76°, and its crystalline aluminum salt, decomp. 270–271°; molecular addition compound of dibenzoylkojic acid and benzoic acid, m.p. 120–121°; 2-(acetoxymethyl)-5-benzoxy-γ-pyrone, m.p. 144°; 2-(benzoxymethyl)-5-hydroxy-γ-pyrone, m.p. 180–181°; and 2-(benzoxymethyl)-5-methoxy-γ-pyrone, m.p. 110–111°. A more convenient synthesis was discovered for 5-benzoxy-2-(hydroxymethyl)-γ-pyrone, and the published melting point was revised from 136° to 143–144°; the melting point of 5-benzoxy-2-(triphenylmethoxymethyl)-γ-pyrone was revised from 206–208° to 213–214°.

INTRODUCTION

Kojic acid, (5-hydroxy-2-(hydroxymethyl)-γ-pyrone) (I), is a crystalline substance which can be produced in good yield by the action of many molds of the *Aspergillus* family on a wide range of carbohydrates. This biological synthesis has been studied extensively because it is the simplest example of the conversion of a sugar to a γ-pyrone. The possibility of utilizing industrial carbohydrate by-products by converting them to kojic acid has also stimulated interest in the chemical properties of this compound. Barham and Smits
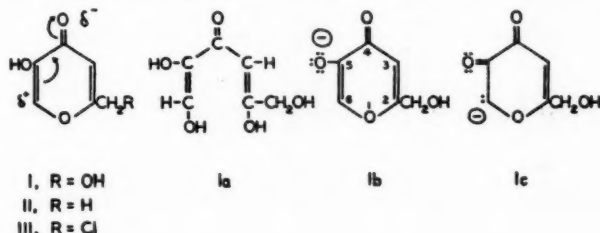
I, R = OH   Ia   Ib   Ic
II, R = H
III, R = Cl

(4) have summarized the earlier literature, and a new review is to appear elsewhere (6).

Practically all the known derivatives of kojic acid were formed by reactions involving the two hydroxyl groups, by nuclear substitutions at position 6, or by the replacement of the ring oxygen atom with the nitrogen of ammonia or amines to give γ-pyridones (6, 11, 12). A few γ-pyrones other than kojic acid, however, were cleaved at the ring oxygen atom by cold dilute aqueous alkalies to yield open-chain derivatives. With the possible exception of Haitinger and Lieben's "xanthochelidonic acid" (10), the dienolic compounds initially produced underwent further changes to more stable ketonic forms (19). Acidification (10), or heating with dilute acid (19), usually regenerated the original γ-pyrone. Willstätter and Pummerer (20) prepared stable derivatives of the dienolic cleavage product of γ-pyrone itself by methylation and benzoylation in the alkaline reaction mixture. The similar open-chain form (Ia) was assumed as the final intermediate in the biosynthesis of kojic acid (6), but the only experimental evidence for its existence was the fact that kojic acid in warm dilute alkali resembled other α-carbonyl dienol structures in reducing Tillman's reagent (2,6-dichlorophenol-indophenol) (18). When the alkali was more concentrated, or was heated, the pyrone ring was fragmented; thus Yabuta (22, 23), and more recently Arnstein and Bentley (3), found that dimethylkojic acid was degraded to formic acid, methoxyacetic acid, and methoxyacetone by boiling aqueous barium hydroxide. The present research had the object of studying the action of bases on kojic acid under milder conditions, and of isolating open-chain derivatives in an unfragmented state.

One-gram samples of kojic acid were accordingly dissolved in approximately 1.2 N sodium hydroxide and the clear yellow solutions were acidified with 20% sulphuric acid after various times. Table I, column 4, records the amount

TABLE I

STABILITY OF KOJIC ACID IN APPROXIMATELY 1.2 N
AQUEOUS SODIUM HYDROXIDE AT 25°

| NaOH, mM.[a] | Water, ml. | Time, hr. | Kojic acid recovered | | | Formic acid, mM. %[a] |
			Fract. 1, gm.	Fract. 2, gm.	Total, %	
2	11.3	1.2	0.76	0.13	89	—
2	11.3	3.0	0.73	0.15	88	—
2	11.3	13.5	0.77	—	—	1.0
2	11.3	32	0.74	—	—	2.5
3	16.9	1.0	0.66	0.19	85	—
3	16.9	32	0.74	—	—	3.2
5	28.2	1.0	0.56	0.25	81	—

[a]Per mM. of kojic acid, 1 gm. or 7.06 mM. of which was used in all cases.

of kojic acid which crystallized when the acidified liquor was kept at −3°; in some experiments the mother liquors were concentrated to recover a second fraction, and in others 60% of the volume was distilled at atmospheric pressure in order to estimate any formic acid produced. Even after 32 hr. in an excess of the alkali, more than 80% of the kojic acid could be recovered as such,

and only a few per cent had been decomposed to formic acid. Kojic acid was therefore either nearly stable in these conditions, or its hypothetical open-chain enolic form (Ia) recyclized almost quantitatively when the solution was acidified.

In attempts to methylate or benzoylate any enolic compound formed, dimethyl sulphate or benzoyl chloride was added to solutions of kojic acid in aqueous alkali. The methylation was carried out with a molar ratio of 1:6:3.7 for kojic acid, potassium hydroxide, and dimethyl sulphate, respectively, but the only product, obtained in low yield, was a new, crystalline monomethyl ether of kojic acid itself. This ether imparted a deep wine color to ferric chloride solution and therefore contained a free phenolic hydroxyl group; methylation with diazomethane gave the known 5-methoxy-2-(methoxymethyl)-γ-pyrone. Hence the new monomethyl ether was 5-hydroxy-2-(methoxymethyl)-γ-pyrone (IV). In the process of purification, IV reacted with the alumina in a chromatograph column to give a pale yellow, crystalline derivative in which three moles were substituted by one atom of aluminum; that is, the derivative had the composition of the aluminum phenoxide. The isomeric monomethyl ether, 2-(hydroxymethyl)-5-methoxy-γ-pyrone (VIII), was obtained in a crude yield of 72% by Campbell and his co-workers (8) from kojic acid, potassium hydroxide, and dimethyl sulphate in a 1:1:1 molar ratio. Heyns and Vogelsang (11) prepared the dimethyl ether from the same reagents, using a molar ratio of 1:8:9.

Two methods were used for the benzoylations, differing mainly in the excess of sodium hydroxide present throughout the reaction. When this excess was considerable, the products were the known, crystalline dibenzoylkojic acid (5-benzoxy-2-(benzoxymethyl)-γ-pyrone) (22, 23) and a new, crystalline addition compound, m.p. 120–121°, between equimolecular amounts of this dibenzoate and benzoic acid. The infrared spectrum of a benzene solution of the adduct was identical with that given by an equimolecular mixture of its compon-
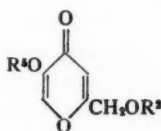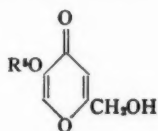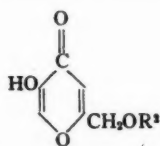
IV R² = methyl
V   R² = benzoyl
VI R² = acetyl
VII R² = trityl

VIII R⁵ = methyl
IX   R⁵ = benzoyl
X   R⁵ = sodium

XI   R² = trityl;   R⁶ = benzoyl
XII R² = benzoyl; R⁶ = methyl
XIII R² = acetyl;   R⁶ = benzoyl

ents, a Perkin-Elmer Model 21 spectrophotometer fitted with a rock salt prism being used for the determination. This addition compound could be recrystallized without change from hydrocarbon solvents, but dissociated into its constituents when dissolved in polar liquids. When the excess of alkali used in the benzoylation was small, the product consisted of the above adduct, together with a monobenzoyl derivative of kojic acid which melted at 142–143°. This melting point was 7° higher than a value reported by Yabuta for a mono-

benzoate prepared from kojic acid and benzoyl chloride in ether (22, 23), or from 5-benzoxy-2-(triphenylmethoxymethyl)-γ-pyrone (XI) by detritylation (25). The latter unequivocal synthesis was repeated, the proper melting point of the resulting 5-benzoxy-2-(hydroxymethyl)-γ-pyrone (IX) was found to be 142–143°, and identity with the monobenzoate under discussion was proved.

Other attempts to isolate a derivative of Ia involved the complete evaporation of aqueous solutions of kojic acid containing one or two molecular equivalents of sodium hydroxide. The product was the new sodium kojate, which occurred either as a crystalline tetrahydrate or in the anhydrous condition. This "salt" was presumably the 5-sodio derivative (X). The tetrahydrate reacted with benzoyl chloride in dry benzene to give the 5-monobenzoate (IX); the yield of the latter rose to 79.5% when anhydrous sodium kojate was used. These observations, together with the results of the methylations and benzoylations, seemed to indicate the absence of an open-chain form like Ia from solutions of kojic acid in dilute aqueous alkali.

As was expected from the inert nature of the carbonyl group, no derivatives could be isolated when kojic acid was treated with hydroxylamine hydrochloride or with semicarbazide hydrochloride. The customary procedures for oximation, however, produced from dibenzoylkojic acid in up to 90% yield a crystalline substance which proved to be a new monobenzoate and not an oxime. Benzoylation in dry pyridine gave back the dibenzoate, and the presence of a free phenolic function in the monobenzoate was indicated by the wine color it produced with ferric chloride. Methylation with diazomethane yielded another new crystalline substance which was identified as 2-(benzoxymethyl)-5-methoxy-γ-pyrone (XII) by a synthesis from VIII and benzoyl chloride. The monobenzoate was therefore the 2-derivative (V).

The selective deacylation displayed by hydroxylamine in the above reaction was also illustrated by the smooth debenzoylation of the 5-benzoxy derivatives of 2-acetoxymethyl- (XIII) and 2-(triphenylmethoxymethyl)-γ-pyrone (XI), and of benzoylallomaltol to the corresponding 5-hydroxy derivatives (VI, VII, and II). The method should thus be useful for preparing derivatives of kojic acid monosubstituted in the generally less reactive 2-position, especially when a substituent such as acetyl or trityl offers but little resistance to conventional methods of hydrolysis. Although the cleavage of esters by hydroxylamine in the presence of a strong base is frequently used for the preparation of substituted hydroxamic acids (26), the method has not hitherto been employed for selective deacylations. Ingold and Shoppee (15), however, incidentally observed that their oximation of 2-benzoxy-4,4,5,5-tetramethyl-2-cyclopenten-1-one (XIV) was accompanied by debenzoylation, although the benzoyl group in the analogous saturated ketone was unaffected under identical conditions. The results support the view that enolic and phenolic ester groups adjacent to a carbonyl group are cleaved with particular ease by hydroxylamine. It therefore seems probable that the same method might prove useful in other compounds containing the same grouping; for example, in the selective deacylation of flavonols at position 3.

Another selective debenzoylation, this time involving the group in the

second instead of the fifth position of dibenzoylkojic acid, was brought about by reduction with zinc dust in glacial acetic acid. The products included nearly one mole of benzoic acid and a crystalline substance which was proved to be the benzoate of allomaltol (II) (5-benzoxy-2-methyl-γ-pyrone). This proof consisted of repeating Yabuta's reduction (24) of 2-(chloromethyl)-5-hydroxy-γ-pyrone (III) to the 2-methyl derivative (II) and benzoylating the latter. The primary alcohol unit in dibenzoylkojic acid thus resembled that in benzyl ethers in its tendency to undergo reductive fission. In accord with this infer-ence, the dibenzoate yielded 0.6 mole of benzoic acid when hydrogenated at room temperature and atmospheric pressure over a platinum catalyst. This hydrogenation probably involved hydrogenolysis, since hydrolysis was not likely in the glacial acetic acid used as a solvent. The other product of the hydrogenation was a sirup which was not studied in detail because numerous earlier catalytic hydrogenations of kojic acid, its ethers and esters, gave sirups of indefinite composition (6). Ingold and Shoppee (15) observed that the enolic benzoyl group in (XIV) was unaffected by zinc or sodium amalgam in glacial acetic acid, while the double bond was readily reduced (with retention of the benzoyl group) by hydrogenation.

Dibenzoylkojic acid failed to form a benzoylhydrazone and a 2,4-dinitro-phenylhydrazone, but condensation occurred with semicarbazide hydrochloride in boiling ethanol–pyridine. The product consisted of two white, neutral, microcrystalline isomers, but the one with the lower melting point was formed in too small yield to be examined in detail. The molecular formula of these isomers, $C_{22}H_{22}N_6O_7$, corresponded to disemicarbazones of an open-chain form of dibenzoylkojic acid, and structures such as XV were tentatively assigned to them. The higher-melting isomer was readily saponified by cold dilute alkali,
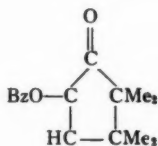
XIV
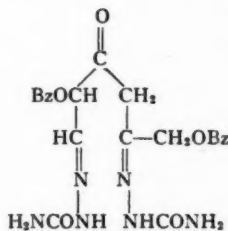
XV

but the only product identified was benzoic acid in nearly quantitative yield. These structures were unusual, for the initial open-chain products from other γ-pyrones and hydrazines invariably lost one additional molecule of water to give cyclic end products. Bedekar and co-workers (5) represented these end products as pyridones, but Ainsworth and Jones (1) recently found very good evidence in favor of pyrazole structures. No carboxylic acid derived from a pyridone or pyrazole, however, could be recovered when the higher-melting isomer was oxidized with potassium permanganate, only benzoic and benzoyl-

glycolic acids being identified. When the same isomer was boiled in 10% hydrochloric acid, the yellow-white, microcrystalline substance which formed in small yield had the molecular formula $C_{21}H_{15}N_3O_5$, which corresponded to the loss of one semicarbazide unit and two molecules of water. Cyclization probably occurred in this case, but the point could not be definitely proved owing to lack of material.

The higher-melting "disemicarbazone" lost one semicarbazide group on treatment with nitrous acid (9, 21), and two neutral, microcrystalline "mono-semicarbazones" resulted. The lower-melting, or "β", form was the main product (69%), and the "α-isomer" was isolated only when a larger quantity of nitrous acid was employed. Since an excess of nitrous acid did not convert the β- to the "α-monosemicarbazone" the two were perhaps position isomers. To judge from the composition of these two products, $C_{21}H_{19}N_3O_7$, both were open-chain derivatives and no cyclization occurred during the treatment with nitrous acid. No carboxylic acids other than benzoic and benzoylglycolic could be isolated when the β-isomer was oxidized with aqueous potassium permanganate. An attempt to regenerate the "disemicarbazone" from the "β-mono-semicarbazone" by treating the latter with semicarbazide hydrochloride and pyridine in boiling methanol failed, the starting material being recovered unchanged. Hydroxylamine hydrochloride likewise had no effect. These results were surprising, because the presence of a carbonyl group created by the fission of a semicarbazone unit could reasonably have been assumed (21); the negative outcome with hydroxylamine also suggested that neither of the two benzoyl groups was of the phenolic type present in dibenzoylkojic acid. At this point, attempts to elucidate the structures of the "monosemicarbazones" had to be abandoned.

The failure of N sodium hydroxide to open the ring of kojic acid stood in sharp contrast to the apparent cleavage of the ring in the dibenzoate by semicarbazide, a much weaker base. Cleavage of γ-pyrone rings was presumably initiated by a nucleophilic reagent at position 6, where the drift of electrons toward the carbonyl group would create an electrophilic center. This might happen in the case of dibenzoylkojic acid, as indicated in I, when semicarbazide was the attacking nucleophile (14). In sodium hydroxide, kojic acid would be present as the kojate anion (Ib), and an important contribution to the resonance hybrid of this anion could be expected from the diketo form (Ic) with the full negative charge located at position 6. This nucleophilic center would face the nucleophilic hydroxyl ion, and no cleavage would result. If this explanation was correct, the production of open-chain forms of kojic acid by basic cleavage required either the use of very weak bases or the blocking of the hydroxyl group in position 5 by a substituent stable to alkali.

EXPERIMENTAL

All melting points were corrected. All of the nitrogen analyses, and two carbon and hydrogen analyses marked by an asterisk, were by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y., U.S.A. Reactants were always weighed to the nearest 10 mgm.

5-Hydroxy-2-methoxymethyl-γ-pyrone (IV) and Its Aluminum "Salt"

Dimethyl sulphate, 47 gm. (0.373 mole), was slowly added over a period of three hours and at room temperature to a stirred solution of 15 gm. (0.106 mole) of kojic acid and 37.5 gm. (0.6 mole) of potassium hydroxide in 315 ml. of water. The mixture slowly warmed to 37°, but was then kept below 30°. The alkaline liquor, having been concentrated at 50° *in vacuo* to 250 ml., was extracted continuously with benzene to remove any dimethylkojic acid, but the yellow extract yielded only traces of an oil. The aqueous liquor was then acidified to pH 2 with 50% sulphuric acid and was re-extracted continuously with benzene for 21 hr. This dark red extract was treated with activated carbon, concentrated to small volume, and diluted with ligroin. The red oil which separated solidified on chilling to a yellow-brown substance, 6.85 gm. melting between 55° and 66°. Crystallization from ethyl acetate left 1.85 gm. (11.2%), m.p. 75–76°, unchanged by recrystallization from toluene–hexane. Found: C, 53.7, 53.8; H, 5.3, 5.2; OCH_3, 20.4, 20.4%. Calc. for $C_6H_5O_3(OCH_3)$: C, 53.9; H, 5.1; OCH_3, 19.9%.

The combined mother liquors from the above crystallizations were evaporated, and a concentrated solution of the residue in benzene was applied to a short column of alumina. Elution with benzene, evaporation of the eluates, and recrystallization of the orange products from ethanol gave 0.71 gm. (4%) of pale yellow crystals melting with decomposition at 270–271°. A qualitative test demonstrated the presence of aluminum. Found: Ash, 10.4; Al, 5.5; OCH_3, 18.6%. Calc. for Al $[C_6H_4O_3(OCH_3)]_3$: Ash, 10.4; Al, 5.5; OCH_3, 18.9%.

A solution of 0.15 gm. of these crystals in water was adjusted to pH 2 with dilute sulphuric acid and evaporated cautiously to a sirup. Extraction of this sirup with toluene yielded 0.09 gm. (63%) of 5-hydroxy-2-(methoxymethyl)-γ-pyrone with the proper melting point, undepressed by admixture with an authentic sample.

A 0.5 gm. sample of the monomethyl ether was kept dissolved in 20 ml. of dry benzene containing about 0.56 gm. of diazomethane (2) for 12 hr. at room temperature. Evaporation of this solution, and crystallization of the yellow residue from benzene–hexane, yielded 0.33 gm. (60.5%) of white needles melting at 89–90°, undepressed by admixture with an authentic sample of dimethylkojic acid (11).

Sodium Kojate and Its Crystalline Tetrahydrate

A solution of 3.0 gm. (21 mM.) of kojic acid and 0.88 gm. (21 mM.) of sodium hydroxide in 15 ml. of water was evaporated to dryness *in vacuo* at 40°. The residual solid was recrystallized twice from 5 ml. of hot water, and the white crystals were dried in air at 25°. Yield, 2.62 gm. or 53%. Found: Na, 10.3; loss of weight at 110°, 30.3, 30.2; at 25° over phosphorus pentoxide, 29.7%. Calc. for $C_6H_5O_4Na.4H_2O$: Na, 9.8; water, 30.5%.

The water of crystallization was determined by drying samples either for about six hours at 110° or for about 18 hr. *in vacuo* over phosphorus pentoxide at 25°. Anhydrous sodium kojate was a white powder when prepared by the latter method, and a tan powder by the former.

A New Synthesis of 5-Benzoxy-2-(hydroxymethyl)-γ-pyrone

Crude sodium kojate was prepared from 2 gm. (14 mM.) of kojic acid as just described, and was rendered anhydrous by drying *in vacuo* over phosphorus pentoxide. The product was shaken in the original tightly-stoppered flask with 50 ml. of dry benzene and 2.5 gm. (18 mM.) of benzoyl chloride at room temperature for 26 hr. It was necessary to interrupt the shaking to loosen solid material which adhered initially to the walls of the flask. After adding 30 ml. of benzene, the white suspension was heated to boiling and was filtered while hot. The residual solid was re-extracted with hot benzene, then with water, and again with benzene. The combined benzene solutions, when dried and evaporated, deposited 2.53 gm. of white needles melting at 143–144°, together with a second fraction which was recrystallized from aqueous acetone to give 0.22 gm. with the proper melting point. Total yield, 79.5%. A mixed melting point with authentic 5-benzoxy-2-(hydroxymethyl)-γ-pyrone (see below) was not depressed.

The authentic sample was prepared from 5-benzoxy-2-(triphenylmethoxymethyl)-γ-pyrone, (XI), which when recrystallized from aqueous dioxane melted at 213–214°, and not at 206–208° as recorded by Yabuta and Anno (25). The removal of the trityl group with boiling 80% acetic acid, followed by the separation of triphenylcarbinol, left a product which was crystallized from benzene. Yield, 55%, and melting point 140–141°. Further recrystallizations from benzene raised the melting point to 142–143°, the value quoted by Yabuta being 135–136°.

Dibenzoylkojic Acid – Benzoic Acid Addition Compound

(a) Benzoylation in Sodium Hydroxide

A solution of 5 gm. (35 mM.) of kojic acid and 7.3 gm. (177 mM.) of sodium hydroxide was prepared in 177 ml. of water. Benzoyl chloride, 19.7 gm. (140 mM.), was added slowly, with stirring and at room temperature, during two and three-quarter hours, and the granular solid which separated was recovered. A solution of 5.8 gm. (140 mM.) of sodium hydroxide in 50 ml. of water was added to the acidic filtrate, and the benzoylation was repeated with another 140 mM. of benzoyl chloride. After one hour more of stirring, a second crop of the solid was recovered from the liquor, which again had become acid. The two crops when combined and crystallized from dry benzene–hexane yielded 1.55 gm. (18%) of a less soluble fraction which consisted of white crystals with the composition and melting point, 142–143°, of 2-(hydroxymethyl)-5-benzoxy-γ-pyrone (IX). A mixed melting point with an authentic sample was not depressed.

The more soluble fraction was composed of 5.88 gm. (35.5%) of matted white needles melting at 117–118°, raised by further recrystallizations to m.p. 120–121°. Mixtures of this molecular addition compound with dibenzoylkojic acid and with benzoic acid showed depressions in melting point. Found: C, 68.8, 69.0; H, 4.4, 4.4%. Calc. for $C_{20}H_{14}O_6 \cdot C_7H_6O_2$: C, 68.6; H, 4.2%.

A solution of 0.50 gm. of the addition compound in 5 ml. of warm acetone was diluted with an equal volume of cold water; the mixture was cooled and the

white precipitate which had immediately formed was recovered. The precipitate melted correctly for dibenzoylkojic acid at 134–135°, a mixed melting point with an authentic sample was not depressed, and the yield of 0.37 gm. was 100% of theory. The clear filtrate when concentrated to half-volume deposited 0.12 gm. (92.5%) of benzoic acid melting at 117–119°, raised to the proper value of 122° by recrystallization from water. A mixed melting point was not depressed. Dibenzoylkojic acid also crystallized from solutions of the adduct in dioxane (on dilution with water), 95% ethanol, ethyl acetate, absolute methanol, and pyridine. The benzoic acid was extracted selectively by aqueous 5% sodium carbonate from a solution of the adduct in benzene. On the other hand, the adduct could be recrystallized substantially without change from benzene, benzene–hexane, toluene, and glacial acetic acid.

(b) Benzoylation in Pyridine

Five grams (36 mM.) of benzoyl chloride was added in several increments to a solution of 2 gm. (14 mM.) of kojic acid in dry pyridine. Next day the pyridine was evaporated in a current of dry air, with gentle heating, to leave a moist pulp. The product in this pulp was separated from the pyridine salts by extraction with hot benzene, and was recovered from the extract in several fractions. The less soluble fractions yielded 3.45 gm. (70%) of pure dibenzoylkojic acid, which was carefully identified as such. Recrystallization of the more soluble fractions from benzene–ligroin gave 0.66 gm. (10%) of white matted needles whose melting point of 120–121° was not depressed by admixture with the dibenzoylkojic acid – benzoic acid addition compound.

The product from a similar benzoylation was isolated in the customary way by pouring the mixture into chilled 5% aqueous sulphuric acid. A 98.5% yield of pure dibenzoylkojic acid was obtained.

2-(Benzoxymethyl)-5-hydroxy-γ-pyrone (V)

A solution of 0.30 gm. (0.86 mM.) of dibenzoylkojic acid and 0.12 gm. (1.7 mM.) of hydroxylamine hydrochloride in 4 ml. of pyridine was kept for 12 hr. at room temperature, then diluted with 20 ml. of cold water. After the suspension had been kept cold for a few hours, the white precipitate was recovered. Yield, 0.19 gm. (90%); m.p. 179–180°, increased to 180–181° by recrystallization from ethanol. Found: C, 63.5, 63.6; H, 4.1, 4.2; COC_6H_5, 41.7, 41.0%. Calc. for $C_6H_5O_4(COC_6H_5)$: C, 63.4; H, 4.1; COC_6H_5, 42.7%.

The same product was obtained in 83% yield by boiling dibenzoylkojic acid with three equivalents each of hydroxylamine hydrochloride and pyridine in 95% ethanol for 30 min.; also by replacing the pyridine with sodium acetate and using 80% ethanol. In the latter case the pure product crystallized in 74% yield when the solution cooled. Three equivalents of either sodium acetate or hydroxylamine hydrochloride alone had no appreciable effect on dibenzoylkojic acid in boiling ethanol.

A 15% excess of benzoyl chloride was slowly added to an ice-cold solution of the above monobenzoate in dry pyridine. An 89% yield of dibenzoylkojic acid was recovered, melting at 135°, after crystallization from aqueous acetone. A mixed melting point was undepressed.

2-(Benzoxymethyl)-5-methoxy-γ-pyrone (XII)

One gram (4.1 mM.) of finely powdered 2-(benzoxymethyl)-5-hydroxy-γ-pyrone was added to 18 ml. of benzene containing about 0.5 gm. of diazomethane (2). Practically all of the solid dissolved within six hours, and the product was isolated by concentrating and cooling the solution. The yield was 0.52 gm. (49%) of near-white crystals melting at 109–110°; the use of absorbent charcoal in ethanol, and recrystallization from benzene–ligroin, gave a snow-white product melting at 110–111°. Found: C, 64.7, 64.9; H, 4.6, 4.8; OCH₃, 12.0, 12.1%. Calc. for $C_{13}H_9O_4(OCH_3)$: C, 64.6; H, 4.6; OCH₃, 11.9%.

The same product was obtained when 2-(hydroxymethyl)-5-methoxy-γ-pyrone (VIII), prepared according to Campbell and co-workers (8), was esterified with benzoyl chloride in dry pyridine. A mixture of the two samples showed no depression in melting point.

2-(Acetoxymethyl)-5-benzoxy-γ-pyrone (XIII)

A solution of 2.8 gm. (11 mM.) of 5-benzoxy-2-(hydroxymethyl)-γ-pyrone (IX) in 24 ml. of dry pyridine was chilled, mixed with 4.1 gm. (40 mM.) of acetic anhydride, and kept overnight at room temperature. Precipitation of the solution into 200 ml. of cold water yielded 3.06 gm. (93%) of crystals melting at 143–144°. Recrystallizations from benzene and from aqueous acetone left the melting point of the white plates at 144°, and a mixed melting point with the starting material was depressed to 120–127°. Found: C, 62.8, 62.5; H, 4.3, 4.5%. Calc. for $C_{15}H_{12}O_6$: C, 62.5; H, 4.2%.

A solution of 0.96 gm. (3.3 mM.) of XIII and 0.46 gm. (6.6 mM.) of hydroxylamine hydrochloride in 13 ml. of pyridine was kept at room temperature for 12 hr. before being diluted with six volumes of cold water. After being extracted with benzene, the aqueous liquor was evaporated to dryness *in vacuo* at 50° and the solid, yellow residue was extracted with boiling benzene. The combined benzene extracts yielded a total of 0.47 gm. (77%) of white needles melting at 134–135°, plus a second fraction (6%) of slightly less pure material. 2-(Acetoxymethyl)-5-hydroxy-γ-pyrone (VI), whose published melting point was 133.5° (12) and 137° (17), gave a wine color with aqueous ferric chloride. When acetylated in the presence of sodium acetate (13), the substance yielded 5-acetoxy-2-(acetoxymethyl)-γ-pyrone which was carefully identified by comparison with an authentic sample.

Debenzoylation of 5-Benzoyl-2-(triphenylmethoxymethyl)-γ-pyrone (XI)

The debenzoylation was accomplished as just described, except that an 8.4 molar equivalent of hydroxylamine hydrochloride was used. After recrystallizations from benzene–ligroin, dioxane–water, and carbon tetrachloride – isopropyl ether, 66% of pure 5-hydroxy-2-(triphenylmethoxymethyl)-γ-pyrone (VII) remained, melting at 180–182° (25).

Benzoylallomaltol from Dibenzoylkojic Acid

Thirty grams of technical grade zinc dust was added to a hot solution of 5 gm. (14.3 mM.) of dibenzoylkojic acid in 125 ml. of glacial acetic acid; the mixture was vigorously stirred on the steam bath, and eight drops of concen-

trated sulphuric acid were added during five minutes. Stirring was stopped after one hour, the hot mixture filtered, the residual zinc rinsed with hot glacial acetic acid, and the combined liquors were evaporated completely *in vacuo*. The residual brown oil was treated with an excess of solid sodium carbonate, the resulting mixture was extracted with boiling benzene, and the benzene extract was washed with dilute aqueous sodium carbonate and with water. Pure benzoic acid, 1.31 gm. (75%), was recovered from the benzene-insoluble solid residue and the sodium carbonate washings. The golden brown benzene solution was completely evaporated and the residual tan solid was recrystallized twice from isopropyl ether, using a small amount of decolorizing charcoal. The yield was 1.48 gm. (45%) of benzoylallomaltol as off-white, flat needles melting at 125–126°. An analytical sample, recrystallized from the same solvent, melted at 126–127°, undepressed when mixed with an authentic sample (24). Found: C, 67.9*, 67.8; H, 4.8*, 4.8%. Calc. for $C_{13}H_{10}O_4$: C, 67.8; H, 4.4%.

A solution of 2.4 gm. (10 mM.) of benzoylallomaltol and 0.95 gm. (14 mM.) of hydroxylamine hydrochloride in 25 ml. of pyridine was kept at room temperature for 11 hr., and was then evaporated *in vacuo*. After being dried over solid potassium hydroxide in a vacuum desiccator, the residual brown oil was extracted several times with boiling benzene. The combined benzene extracts yielded a total of 1.05 gm. of crude allomaltol as white crystals melting at 143–149°; recrystallization from a mixture of isopropyl ether and ethyl acetate left 0.93 gm. (71%) melting at 150–151°. This substance was soluble in water and gave a wine color with ferric chloride. Benzoylation with benzoyl chloride in dry pyridine gave back benzoylallomaltol. An authentic sample of allomaltol prepared by Yabuta's method (24) also melted at 150–151°, not at 166° as reported, and a mixed melting point was undepressed. The present value confirmed that recently given by Looker and Okamoto (16).

Dibenzoylkojic Acid "Disemicarbazone"

(a) A mixture of 14 gm. (40 mM.) of dibenzoylkojic acid, 14 gm. (125 mM.) of semicarbazide hydrochloride, 14 ml. (174 mM.) of pyridine, 40 ml. of water, and 350 ml. of ethanol was boiled under reflux for 50 min. and then kept at room temperature for six days. The crop of yellow-white crystals deposited from the clear solution was extracted in succession with hot ethyl acetate, water, and dioxane, and the residual microcrystalline white solid, 6.04 gm., melted at 212° with decomposition. A second crop, recovered from the original mother liquor 15 days later, increased the yield to 7.96 gm. or to 41.5%. This "disemicarbazone" was sparingly soluble in glacial acetic acid but insoluble in all other liquids tried. The sample for analysis was dissolved in much glacial acetic acid by warming to not more than 70° and was reprecipitated by adding five volumes of cold water. The melting point was then 215° with decomposition. Found: C, 54.8, 54.8; H, 4.7, 4.8; N, 16.9%. Calc. for $C_{22}H_{22}N_6O_7$: C, 54.8; H, 4.6; N, 17.4%.

(b) The above condensation was repeated with 5 gm. of dibenzoylkojic acid, 3.2 gm. of semicarbazide hydrochloride, 5 ml. of pyridine, 10 ml. of water, and

60 ml. of ethanol, the molar ratio of the first three substances being about 1:2:4 instead of about 1:3:4. The yellow solution deposited a yellow-white solid decomposing at 179–181° when concentrated on the steam-bath, and a soft solid separated when the mother liquor was diluted with water and chilled. Extraction of the latter fraction with boiling ethyl acetate removed 0.33 gm. of a solid, m.p. 183–184°, which was combined with the previous fraction of similar melting point. These fractions were purified by extraction with hot ethyl acetate and water to yield 1.65 gm. (24%) of the "disemicarbazone" noted in (a) and melting at 213°.

On cooling overnight, the red ethyl acetate extract deposited 0.61 gm. of white crystals melting at 121° with decomposition. Recrystallizations from ethyl acetate and from ethanol–methanol left 0.4 gm. (6%) of this isomeric "disemicarbazone" and raised the melting point to 172–172.5° with decomposition. Found: C, 54.6, 54.5; H, 5.0, 4.9; N, 16.6%. Calc. for $C_{22}H_{22}N_6O_7$: C, 54.8; H, 4.6; N, 17.4%.

Experiments with the Higher Melting "Disemicarbazone"

(a) Action of Dilute Alkali

Two grams of the above "disemicarbazone" dissolved almost completely when stirred at room temperature for 30 min. with 20 ml. of 10% potassium hydroxide. The clear filtrate contained no substances that could be extracted by ether or chloroform. When acidified with hydrochloric acid, the filtrate deposited 0.95 gm. (95%) of benzoic acid with the correct melting point and mixed melting point.

(b) Action of Dilute Acid

A suspension of 1.5 gm. of the "disemicarbazone" in 25 ml. of 10% hydrochloric acid was boiled under reflux for three and one-half hours. The sticky brown residue was separated from the acidic filtrate (which yielded 0.31 gm., or 41%, of benzoic acid) and was crystallized from methanol–ethanol–benzene, from dioxane–water, and from acetone. The pure product, 0.11 gm. or 9%, was a white, microcrystalline solid melting at 244°. Found: C, 64.3, 64.5; H, 4.1, 4.2; N, 10.8%; mol. wt. (Rast), 423. Calc. for $C_{21}H_{15}N_3O_5$: C, 64.8; H, 3.9; N, 10.8%; mol. wt. 389.

(c) Oxidation with Potassium Permanganate

A mixture of 5.61 gm. (11.6 mM.) of the "disemicarbazone", 10 gm. (63 mM.) of potassium permanganate, 4 ml. (70 mM.) of glacial acetic acid, and 370 ml. of water was kept at 90° for 30 min. After removing the manganese dioxide, the weakly acid filtrate was concentrated to 250 ml. in vacuo, was adjusted to pH 2, and was continuously extracted with benzene. The residue from the extract, when fractionally crystallized from benzene–hexane, yielded 0.38 gm. (27%) of pure benzoic acid, and 0.24 gm. (11.5%) of benzoylglycolic acid melting correctly (7) at 111–112°. Found for the latter: C, 60.0; H, 4.8%*; neut. equiv., 179.7. Calc. for $C_9H_8O_4$: C, 60.0; H, 4.4%; neut. equiv., 180.

Dibenzoylkojic Acid "α-Monosemicarbazone"

A suspension of 3.05 gm. (6.33 mM.) of the "disemicarbazone" in 70 ml.

of glacial acetic acid was stirred at room temperature, while a concentrated aqueous solution of 10.8 gm. (157 mM.) of sodium nitrite was added very slowly during six hours. Stirring was discontinued one hour later, and next day the yellow-green solution was filtered from 0.3 gm. of the starting material. Dilution of the filtrate with an equal volume of cold water precipitated 2.03 gm. of a white solid melting at 173–174°. This solid contained several components, but only one could be isolated in a pure form by recrystallizing the mixture from benzene–methanol (1 vol. : 4 vol.). The product, 0.24 gm. (9%), was a white microcrystalline powder melting with decomposition at 212–213°, increased to 215° by recrystallizations from acetone–methanol. A mixed melting point determination with the original "disemicarbazone" (m.p. also 215°) was markedly depressed. Found: C, 59.6, 59.0; H, 4.7, 4.6; N, 10.1%. Calc. for $C_{21}H_{19}N_3O_7$: C, 59.3; H, 4.5; N, 9.9%.

Dibenzoylkojic Acid "β-Monosemicarbazone"

The preparation for the α-isomer was repeated with 7.2 gm. (104 mM.) of sodium nitrite instead of 10.8 gm., and with 60 ml. instead of 70 ml. of glacial acetic acid. The filtered liquor on dilution with 240 ml. of cold water deposited 2.41 gm. of a white solid decomposing at 166–170°. This product was extracted with 120 ml. of boiling methanol to remove a small amount of unchanged "disemicarbazone". The filtered extract, when diluted with 500 ml. of water, deposited 1.82 gm. (69%) of a white, microcrystalline solid whose decomposition point at 178–179° was not altered by recrystallization from benzene–methanol–ligroin. Found: C, 59.3, 59.2; H, 5.1, 4.9; N, 9.8%. Calc. for $C_{21}H_{19}N_3O_7$: C, 59.3; H, 4.5; N, 9.9%. A sample was recovered unchanged after its suspension in glacial acetic acid had been treated with an excess of aqueous sodium nitrite at room temperature.

Another sample, 0.60 gm. (1.4 mM.), was heated on the steam bath with 1.1 gm. (7 mM.) of potassium permanganate and 40 ml. of water for one and three-quarter hours. The reaction mixture, when worked up as already described for the oxidation of the "disemicarbazone", yielded 0.05 gm. (19.5%) of benzoylglycolic acid melting at 106–110°. Recrystallization raised the melting point to 111–112°, undepressed by admixture with the previous sample. The other product of the oxidation was benzoic acid, which was recrystallized until pure.

REFERENCES

1. AINSWORTH, C. and JONES, R. G. J. Am. Chem. Soc. 76: 3172. 1954.
2. ARNDT, F. Organic syntheses. Vol. XV. John Wiley & Sons, Inc., New York. 1935. p. 4, Note 7.

3. ARNSTEIN, H. R. V. and BENTLEY, R.   J. Chem. Soc. 3436.   1951.
4. BARHAM, H. N. and SMITS, B. L.   Trans. Kansas Acad. Sci. 37: 91.   1934.
5. BEDEKAR, D. N., KAUSHAL, R. P., and DESHAPANDE, S. S.   J. Indian Chem. Soc. 12: 465.
    1935.
6. BEÉLIK, A.   In Advances in carbohydrate chemistry. Vol. 11.   Edited by M. L. Wolfrom.
    John Wiley & Sons, Inc., New York.   To be published in 1956.
7. BRIGL, P. and GRÜNER, H.   Ber. 65: 641.   1932.
8. CAMPBELL, K. N., ACKERMAN, J. F., and CAMPBELL, B. K.   J. Org. Chem. 15: 221.   1950.
9. GOLDSCHMIDT, S. and VEER, W. L. C.   Rec. trav. chim. 65: 796.   1946.
10. HAITINGER, L. and LIEBEN, A.   Monatsh. 5: 339.   1884.
11. HEYNS, K. and VOGELSANG, G.   Ber. 87: 1377.   1954.
12. HEYNS, K. and VOGELSANG, G.   Ber. 87: 1440.   1954.
13. HURD, C. D. and SIMS, R. J.   J. Am. Chem. Soc. 71: 2440.   1949.
14. INGOLD, C. K.   Structure and mechanism in organic chemistry. Cornell Univ. Press,
    Ithaca, N.Y.   1953.   pp. 206, 214.
15. INGOLD, C. K. and SHOPPEE, C. W.   J. Chem. Soc. 1868.   1928.
16. LOOKER, J. H. and OKAMOTO, T. T.   Abstr. of Papers, Am. Chem. Soc. 125: 9N.   1954.
17. MAURER, K.   Ber. 63: 25.   1930.
18. PETUELY, F. and KÜNSSBERG, U.   Monatsh. 83: 80.   1952.
19. SCHÖNBERG, A. and SINA, A.   J. Am. Chem. Soc. 72: 1611.   1950.
20. WILLSTÄTTER, R. and PUMMERER, R.   Ber. 38: 1461.   1905.
21. WOLFROM, M. L.   Rec. trav. chim. 66: 238.   1947.
22. YABUTA, T.   J. Chem. Soc. Japan, 37: 1185.   1916.   Chem. Abstr. 17: 1475.   1923.
23. YABUTA, T.   J. Chem. Soc. Japan, 37: 1234.   1916.   Chem. Abstr. 17: 1475.   1923.
24. YABUTA, T.   J. Chem. Soc. 125: 575.   1924.
25. YABUTA, T. and ANNO, K.   J. Agr. Chem. Soc. Japan, 23: 104.   1949.   Chem. Abstr.
    44: 3492.   1950.
26. YALE, H. L.   Chem. Revs. 33: 209.   1943.

Contents